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METHODOLOGY INVESTIGATION FINAL REPORT

RAPID EVALUATION OF ENVIRONMENTAL HAZARDS: THE FATE OF VX AND GB IN THE DUGWAY PROVING GROUND ENVIRONMENT

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VX persisted in DPG test area soil for less than 2 weeks at 20 °C and a wind of 4 mph (6.4 kph). At 40 °C VX persisted for 1 or 2 days. Temperature and wind were the two environmental factors that produced the greatest effect upon the decay of VX. VX decay in surface water was rapid at 40 °C and moderately slow at 10 °C. At 40 °C VX disappeared in 1 or 2 days; at 10 °C the agent persisted for several months. Surface water in the test area (temporary ponds) can reach 20-30 °C during the warmer months. Thus, any VX contamination would be of short duration.

VX persisted in growing plants for approximately 3 weeks after topical contamination. It was taken up by plant roots and rapidly translocated to stems and leaves. VX also persisted in dead, dry vegetation for approximately 3 weeks at 10 °C and 2 days at 40 °C.

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Desert Soil Moisture
Desert Soil Composition
Residual Toxic Hazard
Hazard Prediction
Extraction of Agent
Radiotagged Agents
Agent Analyses
Agent Decomposition Products
VX Decomposition in Soil
GB Decomposition in Soil
VX Decomposition in Water
GB Decomposition in Water
Agent Contamination of Plants
Decomposition of Agent in Plants
Environmental Impact of Agents
Identification of Agents GB and VX
Identification of Agents GB and VX Decomposition Products

BLOCK 19 (ABSTRACT)

GB was rapidly lost from soil and dry vegetation. GB persisted only 10-12 hours at 40 °C with a gentle breeze (4 mph). At 10 °C GB persisted for less than 4 days with a gentle breeze. Below 0 °C, the agent will persist much longer (several weeks). Environmental factors which had the greatest effect upon the disappearance of GB were windspeed and temperature. Moisture content and agent contamination level had lesser effects.

GB disappeared rapidly from growing plants. This is true for bean plants and desert plants growing at DPG. Contamination of these plants usually lasted less that 1 day, regardless of the route of contamination (topical or uptake).

GB disappeared from surface water in less than a week, even at 10 °C when the initial concentration of GB in water was 10 μg/mL or less. This concentration of GB is greater than what would be expected at surface water locations at DPG. GB decomposition rate was strongly dependent on the presence and concentration of cations such as calcium, sodium, etc. Experiments conducted with distilled water indicated a much longer lifetime for GB than experiments with DPG surface water.

The soil persistence of malathion was compared to the soil persistence of GB and VX. Although the nerve agents are approximately 10,000 times as toxic as malathion, after a relatively short time on soil the residual toxic hazard from malathion can be many times greater than that from GB or VX.

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FOREWORD

This report describes an investigation conducted by the Chemical Laboratory Division, U.S. Army Dugway Proving Ground, Utah during the periods 1969-1974 and 1981-1984. The early portion of this investigation was conducted and funded under the title of Program SAFEST. Although most of the experimental data were obtained during 1969-1974, the investigation was incomplete. Funding by the U.S. Army Test and Evaluation Command under the Methodology Improvement Program was received in June 1981. The investigation was re-initiated as Rapid Evaluation of Environmental Hazards (Binary), Improved Binary Agent Persistence and Decay, TRMS No. 7-CO-PB1-DP1-004. This methodology funding permitted completion of the experimental work and preparation of the report.

The basic laboratory data, on file in the Chemical Laboratory Division, includes laboratory notebooks 485, 766, 771, 773, 792, 841, and 851.

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SECTION 1. SUMMARY

1.1 BACKGROUND

In 1968, U.S. Army Dugway Proving Ground (DPG), Utah scientific staff prepared a comprehensive plan to study the fate of chemical nerve agents in the environment. This plan consolidated all aspects of toxicology, ecology, meteorology, test technology, test area surveillance, disaster control, public relations, etc. The program was approved for implementation by the Secretary of the Army in July 1969. This program plan was named SAFEST and was used as a research plan in subsequent investigations.

Shortly after Program SAFEST was started, the Secretary of the Army established an "Interagency Ad Hoc Advisory Committee for Review of Testing Safety at DPG". This committee, chaired by the Surgeon General, US Public Health Service, reviewed the SAFEST plan and in their report dated November 1968 (reference 1) gave overwhelming support to the program. In addition, they recommended considerable amplification of the research projects and strongly recommended that the fate of chemical agents in the Dugway environment be thoroughly investigated. Before the environmental impact of testing chemical munition systems could be established, more sensitive extraction and analysis methods for agents GB and VX (see Glossary, Appendix C for the identity of the Chemical Symbols) and their related residues had to be developed and their reliability established. Previous methods used to detect and quantitate VX and GB were satisfactory at levels of military significance. However, the desired detection levels were two or three orders of magnitude below the military significance levels.

Successful development of more sensitive extraction and analytical procedures allowed starting a series of laboratory studies with the objective of establishing the fate and/or persistence of VX and GB in samples of soil, vegetation, and water from in and around DPG. The first studies were conducted with GB (reference 2). Also, studies were conducted in which the uptake, translocation and elimination of GB by growing plants were studied (reference 3). A second laboratory study dealt with VX decay in soil, water, and dry vegetation. Plant uptake and topical contamination experiments were also included in this study. The soil and dry vegetation investigation (GB and VX) were closed system experiments (samples were contained in stoppered flasks). This arrangement permitted studying temperature effects, moisture content, contamination level, and the interaction of these factors upon agent decay. In addition, the information obtained from these experiments closely approximating the decay of VX and GB in soil below the surface. However, windspeed could not be included as one of the factors

if the moisture content of the sample was to be maintained at a particular level. This design would approximate the decay of the agents upon the soil surface. Subsequently, an environmental chamber was acquired that permitted the inclusion of windspeed as a factor while maintaining the temperature and moisture content of a sample at a selected level. Another series of experiments were designed to study the decay of VX and GB in samples held in the environmental chamber. Because the environmental chamber could vary factors over a selected range, a more accurate estimate of agent decay in the DPG environment was obtained than was achieved using the closed flask design. In addition, a limited number of experiments were run using the pesticide malathion in order to compare agent persistence with this commonly used pesticide.

Although many portions of the above experiments were completed, budgetary constraints and higher priority projects necessitated the termination of Program SAFEST before the data analysis could be completed and the results reported. Recently, this information was needed for the preparation of environmental documentation for the binary M687 and BIGEYE test programs. Also, information about the fate of QL in the DPG environment was needed (QL is a reactant in VX binary munitions). Funds were provided for the VX and GB studies (reference 4), work was re-initiated, and the results reported herein. The fate and persistence of QL in the DPG environment will be reported elsewhere.

1.2 OBJECTIVES

Provide proven experimental methods and data based models for rapid assessment of the environmental impact of Chemical components and products in the DPG environment and to obtain a comparison of residual toxic hazards between VX and GB with a commonly used insecticide malathion.

1.3 SUMMARY OF PROCEDURES

The procedures used in this investigation are too numerous to list here. A complete description can be found in Section 3, Appendix B.

1.4 SUMMARY OF RESULTS

VX persisted in DPG test area soil for less than 2 weeks at a temperature of 20 °C and a wind of 4 miles/hour (mph which is equal to 6.5 kilometer/hour (km/h). At 40 °C the agent will persist for 1 or 2 days. Temperature and wind had the greatest influence on the decay of VX.

The decay of VX from surface water was rapid (1 or 2 days) at 40 °C and moderately slow (several months) at 10 °C. Although the persistence is moderately long at 10 °C, it is felt that most surface water in the test area (temporary ponds) that could become contaminated with VX will warm during the day and can reach temperatures of 20-30 °C, especially during the spring and summer.

VX persisted in dead dry vegetation for approximately 3 weeks at 10 °C and 2 days at 40 °C. VX persisted in growing plants for approximately 3 weeks after topical contamination. The agent was also taken up by the plant roots from contaminated nutrients and rapidly translocated to the aerial sections (stems, leaves).

GB was rapidly lost from soil and dry vegetation and persisted only for 10-12 hours at 40 °C with a gentle breeze (4 mph). At 10 °C the agent persisted for less than 4 days. At temperatures below freezing, the agent persisted much longer (several weeks). The environmental parameters which had the greatest effect upon the disappearance of GB were windspeed and temperature. The moisture content and agent contamination level had lesser effects.

GB disappeared rapidly from growing plants. This was true for bean plants as well as the species indigenous to DPG. The contamination of these plants usually lasted less than 1 day regardless of the route of contamination (topical or uptake).

GB decomposed in surface water in less than a week even at 10 °C when the concentration was 10 μg/mL or less. This concentration of GB is greater than what would be expected in DPG surface water. The rate of GB decomposition was strongly dependent on the presence and concentration of cations such as calcium, sodium, etc. This was shown in experiments conducted with distilled water in which a much longer lifetime for GB was found than when surface water collected at DPG was used.

Soil persistence of malathion (commonly used insecticide) was compared to that of GB and VX. Although the nerve agents are approximately 10,000 times more toxic as malathion, the latter is far more persistent than the nerve agents. After a relatively short time (several days) on soil, the residual toxic hazard from the malathion was many times greater than that from GB or VX.

1.5 ANALYSIS

Numerical results and prediction equations were obtained for the persistence of VX and GB in a number of natural materials (soil, vegetation, water) covering a range of environmental conditions. The experimental designs and analytical methods developed for this work provide a protocol for future chemical persistency studies.

1.6 CONCLUSION

The persistency of VX and GB in samples of natural materials found in the DPG environment is of surprisingly short duration and the agents rapidly decay to a safe level.

1.7 RECOMMENDATIONS

Similar studies should be made as new agents are developed or different geographical locations are selected for testing, storage, or manufacturing.

SECTION 2. DETAILS OF INVESTIGATION

2.1 EXPERIMENTAL

2.1.1 SOIL INVESTIGATIONS

2.1.1.1 Description of Soil Area and Soils Studied

DPG is at the southeastern edge of the Great Salt Lake Desert and within the Great Basin of the western United States. Mountains are on three sides of DPG. The fourth side is bounded by the Great Salt Lake Desert. The mountains are not extensive with the exception of the Cedar Mountains on the northeastern and northern boundaries. A number of isolated mountains are found within the confines of DPG. Each of these mountains is surrounded by gentle sloping piedmonts and separated by level flats. Lake terraces and other evidences indicate that DPG was submerged many times during prehistoric times. Lake Bonneville sediment, modified by wind and water erosion, have largely determined the present composition of the DPG soils.

The soils range from highly alkaline clays to saline loams, sands, and gravels. The DPG area is also known as a salt desert, as described by Steward (reference 5), because the salt content of some soils limits plant distribution. Flowers (reference 6 and 7) and Vest (reference 8) have extensively studied the soil and plant communities at DPG and have classified the soils according to predominating plant species found within eight general areas. Based upon this classification, three surface soils of differing physical and chemical compositions were selected for use throughout this investigation. The soils chosen were: pickleweed, shadscale-gray molly-greasewood, and vegetated dune soils.

Limited investigations were carried out with uncharacterized soils from other localities in the United States.

A. Soil Composition

The description of the soils used in this experiment applies primarily to the top 5 cm of each soil type. Only a small amount of soil is likely to become contaminated at greater depths as a result of testing.

The pickleweed soil is a mineral soil, ranging from a clay to a sandy clay with 30 to 40 percent of the particles less than 0.002 mm. The remainder is 9 to 20 percent silt and 30 to 55 percent sand. Ground-water in the pickleweed area is near the surface and is often drawn to the surface by capillarity and evaporates producing high salt concentrations at the surface. The soluble salt content in the top two inches of the soil is often as high as 21 percent. The salt concentration of pickleweed soil has been determined at various depths (reference 7, 8) and is significantly higher than the salt content at the same depth in the other soil areas. Table 1 compares pickleweed soil with vegetated dune soil.

Table 1. Salt Content of Vegetated Dunes and Pickleweed Soils at Four Depths(8)

(PERCENT)

SOIL AREA	DEPTH	NaCl	Na2SO4	Na2CO3	NaHCO3	Total[a]	pH
Vegetated Dune	5 cm	0.019	0.019	0	0.050	0.154	9.4
	30 cm	0.016	0.026	0	0.052	0.112	9.7
	60 cm	0.027	0.026	0	0.047	0.146	9.7
	90 cm	0.040	0.009	0	0.054	ND[b]	ND
Pickleweed	5 cm	20.80	0.842	0	0.018	21.600	9.1
	30 cm	4.90	0.204	0.021	0.087	5.300	8.7
	60 cm	4.05	0.275	0.037	0.081	4.240	8.6
	90 cm	5.40	0.204	0.023	0.070	5.47	ND

[a]Total soluble salt content expressed as percent of soil composition.

[b]Not determined.

Shadscale-gray molly-greasewood soil is a mineral soil classified as a sandy-clay loam. The soil is primarily a lake

deposite. Up to 50 percent of its texture is sand (particles larger than 0.05 mm). The remainder is silt and clay. The soluble salt content of surface soil from this area is 1.75 percent and increases with depth. Because most of the agent/soil investigations discussed in this report were conducted with the shadscale-gray molly-greasewood soil, its composition is shown in Table 2.

Table 2. Composition of DPG Test Area Soil (Shadscale-Gray Molly-Greasewood).

Soil pH (Sat. paste)	8.92
Water Soluble Salts	1.75 percent
Soluble Chlorides	1.04 percent
Carbonates ($CaCO_3$)	32.9 percent
Clay Fraction	28-29 percent
Organic Content	less than 1.0 percent
Bulk Density	1.4 g/cc
Cation Exchange Capacity	30 meq/100 g[a]

[a]Cation exchange capacity is expressed as milliequivalence/100 grams

Several factors summarized in Table 2 merit further discussion. First, the soil is quite alkaline and has a high salt content. Thus, one of the principle modes of agent decay would be through alkaline hydrolysis. Also, the high salt content limits vegetation growth to salt tolerant plants. Second, the soil has a substantial clay fraction and a moderately high cation exchange capacity. Although the actual clay mineralogy (identification of the clay type) was not determined, a cation exchange capacity of this magnitude indicates the presence of some multiple lattice clays. Multiple lattice clays are often "swellable" and retard the downward migration of surface water or water containing agent. Thus, agent applied to the soil would probably remain near the surface.

Soil from the vegetated dune area is classified as a sand with up to 80 percent of the soil composed of sand, largely quartz. The soluble salt content of this soil is usually less than 0.2 percent. Salt concentrations at various depths of soil are shown in Table 1.

Soil salt content has been emphasized because it influences the distribution of plants within the selected soil areas. This is dramatically demonstrated by the difference in distribution of plants in the pickleweed area (high salt content) and the vegetated dune areas (low salt). The pickleweed area contains primarily one species of plant, Pickleweed [Allenrolfea occidentalis, (S. Wats) Kuntze]. In the vegetated dune areas, a variety of shrubs, ephemerals, and grasses are found. These two soil areas are the soil extremes at DPG. The shadscale-gray molly-greasewood soil is intermediate, and was selected because of its wide occurrence in the DPG test areas.

B. Soil Texture

Soil texture is an important edaphic factor influencing the distribution of plants in the test areas. Soil texture regulates the proportion of the scanty precipitation available to plants at DPG. The coarser soils of the vegetated-dunes are easily penetrated by moisture. It has been reported by Vest (reference 8), that in level dune areas, penetration occurred to over 60 centimeters in depth. This indicates the ease with which water will penetrate into the dune soil and become unavailable for use by most plants. In contrast, water will penetrate the clay soils of the shadscale-gray molly-greasewood area to a depth of only 4 cm. When this clay soil is moistened by rain, the colloids swell and the soil becomes almost impervious to water. Water is lost primarily through evaporation and the area is characterized by scanty vegetation. Most of the plant species in the area are salt tolerant.

C. Soil Moisture Content

To determine the range of soil moisture used for the agent-on-soil experiments, samples of the three soil types were collected during various times of the year. Some soils were collected the day following rain or when snow was present. Known amounts of each soil were placed in weighed petri dishes and vacuum dried at 100 ^{o}C to determine the total moisture content of the soil. No attempt was made to differentiate between capillary and hydroscopic soil moisture content. The results are shown in Table 3.

The high moisture level in the pickleweed soil during the winter and spring is due to the high water table in this area. However, during the summer the water table recedes and the

surface soil becomes quite dry. On a typical June day the moisture content of the surface soil was 2.8 percent, 7 percent at 2.5 cm, and 22.6 percent at 15 cm. The same general trend was obtained in the shadscale-gray molly-greasewood soil. Because the water table is much deeper in the shadscale-gray molly-greasewood area, the soil moisture content was lower at 15 cm than in the pickleweed soil. The moisture content in the vegetated dune soil was fairly uniform at all depths and was different only if samples were collected immediately after a rain storm or snow melt. This uniformity is the result of the ease with which water penetrates the soil.

The moisture content of the surface soil also depended upon the time of day the samples were collected. Most samples were collected routinely at 1400 hours during the course of a year. A few surface soil samples were collected during the morning hours (0830) and had higher moisture content than did samples collected later in the day. For example, samples collected on an August morning in the shadscale-gray molly-greasewood and the vegetated-dune areas yielded surface soil moisture contents of 0.8 and 0.6 percent respectively. Samples collected in the afternoon of the same day yielded surface moisture contents of 0.4 and 0.2 percent respectively. It is conceivable that even higher soil moisture content would exist during the night before the surface soil is warmed by the early morning sun. (The effect of air temperature upon soil surface temperature is discussed in the next section). It was concluded from this information that soil moisture levels of 1 to 21 percent would reasonably bracket the year-round moisture content found in the soil areas. These levels were used in this study.

D. Soil Temperature

DPG is in a middle-latitude dry climate or steppe region characterized by hot dry summers, cool spring and fall, and moderately cold winters. The climate at DPG is discussed by Opstad (reference 9). Relatively severe seasonal temperature extremes and large diurnal ranges of temperature occur. Air temperature extremes, intense sunlight, soil color and scarcity of vegetative cover in most areas at DPG causes the soil surface temperatures to fluctuate over a wide range during the year. In addition, soil surface temperature during mid-day can be significantly higher than the air temperature. Consequently, soil surface temperatures were measured by the Meteorological Branch, DPG. A thermocouple sensor was placed near Tower Grid in the test area of DPG and portable thermographs were located in the pickleweed and the vegetated-dune soil sampling sites.

Table 3. Percent Moisture in Top 0.64 cm of Three Soil Areas at DPG.

Conditions	Pickleweed	Shadscale-Gray Molly-Greasewood	Vegetated Dune
18 Feb 72, mild weather, no precipitation, air temp. 10 degrees C at collection, 1400 hours	20.6	7.9	1.6
6 Jul 72, no precipitation, air temp. 38 degrees C at collection, 1400 hours	0.7	0.9	ND[a]
2 Aug 72, thunder showers previous evening, air temp. 27 degrees C at collection, 1400 hours	2.6	ND	0.6
28 Aug 72, precipitation previous evening, air temp. 27 degrees C at collection, 1400 hours	14.0	2.0	1.4
31 Jan 73, partial snow cover, air temp. 3 degrees C at collection, 1400 hours	14.2	8.7	9.3
1 Mar 73, precipitation previous evening, air temp. 10 degrees C at collection, 1400 hours	22.2	12.9	ND

[a]ND Not determined.

Most field tests have been conducted at DPG between March and October. However, air and soil temperature data were collected from August 1972 to August 1973. This information was used to estimate surface soil temperature at DPG throughout a

year. This information was vital to the agent persistence studies because of the need to select temperature limits to be used in the laboratory studies. Although temperature data were collected on a continuous basis, three days were selected for illustration and represent three seasons at DPG; spring, summer, and early winter. Surface and ambient air temperatures over 24 hours for each of the three days are in Table 4.

Table 4 data illustrate the wide fluctuation in temperature (air, surface) both during the year and during the day. The surface soil temperature in the early spring reached as high as 10 to 20 °C and remained at that level for several hours. When the soil surface temperature reached 20 °C, the ambient air temperature was only 6.1 °C. However, at night the air temperature was often warmer than the ground temperature, especially during the winter. However, it is rare for the soil surface temperature to drop below -5 °C even when the air temperature reaches -26 °C. It was observed during daylight hours in the winter when the ambient air temperature was below zero, many times the ground temperature remained above freezing. As late as the month of November, surface soil's temperatures reached as high as 11 °C and remained there for several hours. In contrast, during the summer months the surface temperatures were often in excess of 54 °C. At the same time, the ambient air temperature reached only 34 °C.

The soil color significantly affected the soil surface temperature. For example, on July 10, 1973, the desert gray soil of the shadscale-gray molly-greasewood area recorded a maximum surface temperature of 53 °C. The yellowish-tan sands found in the vegetated-dune area recorded a surface temperature of 58 °C. However, the white soil in the pickleweed area recorded only a 38 °C maximum temperature.

As a result of this information it was concluded the temperature range of 10 to 40 °C, obtainable in the available environmental chamber, reasonably describes the temperature regime to be found during a large portion of the year at DPG. This temperature range was used in these laboratory studies.

2.1.1.2 Sample Preparation

A. Closed-Flask Experiments

Table 4. Soil Surface and Ambient Air Temperatures in the Shadscale-Gray Molly-Greasewood Soil Area.

Time	26 Nov 1972 Ground (°C)	26 Nov 1972 Air (°C)	24 Mar 1973 Ground (°C)	24 Mar 1973 Air (°C)	19 Jun 1973 Ground (°C)	19 Jun 1973 Air (°C)
0000	0.1	1.8	0.4	1.6	21.5	26.6
0100	0.2	1.8	0.2	0.9	22.1	25.5
0200	-0.3	1.2	0.0	0.1	22.2	25.0
0300	-0.5	1.1	-0.3	0.3	20.8	24.2
0400	-0.8	0.8	-0.5	-0.4	19.8	24.5
0500	-1.1	0.6	-0.7	-0.9	18.5	21.6
0600	-1.1	1.0	-1.0	-0.8	20.6	23.0
0700	-1.0	1.0	-1.0	-0.5	25.2	25.2
0800	-1.1	1.1	-0.1	-0.3	30.7	27.7
0900	-0.6	2.6	ND[a]	ND	27.5	29.0
1000	3.6	4.3	10.2	2.8	32.9	29.1
1100	7.9	6.1	16.4	4.2	44.8	29.1
1200	8.4	7.6	20.1	6.1	48.4	30.4
1300	8.8	8.7	19.6	6.9	49.3	31.0
1400	10.5	10.2	19.5	7.9	52.4	32.2
1500	11.5	11.0	18.5	9.0	54.1	34.2
1600	7.7	8.6	16.5	9.4	49.5	33.8
1700	5.4	6.7	13.3	9.3	42.3	33.4
1800	3.8	5.9	9.5	9.3	34.4	30.9
1900	2.9	6.5	5.8	7.0	32.2	30.9
2000	3.3	5.6	3.7	6.2	28.8	29.5
2100	3.5	6.2	2.3	5.0	27.6	28.8
2200	4.3	7.5	1.0	3.0	25.4	28.3
2300	4.0	5.5	0.5	4.3	24.4	27.6

[a]Not determined.

(1) VX experiments

The soil sample collected from the field was air dried in the laboratory, mixed, and screened. The soil fraction that passed thru a 20 mesh screen and was retained on a 60 mesh screen was used. Weighed portions of the soil were placed in large Erlenmeyer flasks and the moisture contents adjusted with distilled water, thoroughly mixed, and equilibrated by shaking

for several hours followed by standing for several days. A series of samples was prepared from these moisture-adjusted soils by weighing 10 grams of soil into 125 mL Erlenmeyer flasks. Appropriate quantities of VX dissolved in 2-propanol were added to the soil samples to produce the desired concentrations of VX in the soil (Concentrated solutions of VX in 2-propanol, 10,000 and 100,000 μg/mL, were used to minimize the volume of alcohol). The samples were stoppered, immediately mixed, stored at the desired temperature, and extracted at predetermined time intervals. Untreated soil samples were extracted at the same time and used as the analytical blanks.

(2) GB experiments

The GB closed-flask experiments have already been described in detail (reference 2). Summaries of that work will be included in this report for completeness. The GB samples were prepared essentially as described above for the VX samples; in fact, the VX procedures were based on the work with GB, which preceded the VX experiments.

B. Open-Container Experiments

(1) VX experiments

The soil samples were air dried and prepared as described for the closed system. Three gram portions of soil were weighed into 54 mm petri dishes. These petri dishes were placed into the environmental chamber and allowed to equilibrate (usually one day) at the pre-set relative humidity and temperature in order to obtain the desired soil moisture content. In addition to the petri dish soil samples, a second set was prepared by weighing 3 gram portions of test area soil into 125 mL Erlenmeyer flasks. The flasks also were placed in the environmental chamber, unstoppered and allowed to equilibrate in order to obtain the desired soil moisture content.

After the soil samples had equilibrated, the samples (both petri dish and flask samples) were contaminated by applying measured volumes of concentrated solutions of VX in 2-propanol as small droplets to the surface of each sample. The petri dish samples were placed in a miniature wind tunnel within the chamber. This arrangement allowed for passage of air across the surface of the soil samples. The Erlenmeyer flasks also were placed in the environmental chamber and were left unstoppered. The Erlenmeyer samples were considered zero wind speed samples.

(2) GB experiments

The same techniques were used to prepare the samples for the open-container GB experiments as were used for VX and for the GB closed-flask experiments.

2.1.1.3 Experiment Design

A. Closed-Flask Experiments

(1) VX on Soil

Factorial experiment designs were used because of their maximal efficiency (allow reducing the number of samples needed to obtain the desired information compared to vary one-factor-at-a-time experiments) and precision, and because they minimize erroneous conclusions caused by interaction between factors.

The rate of decay of VX in test area soil in closed flasks was determined using a 3x3x2 experiment having the following factors and levels: soil temperatures of 10, 25, and 40 ^{o}C; initial agent concentrations of 1, 100, and 1,000 micrograms VX/g soil; and soil moisture contents of 1 and 11 percent. A complete set of samples consisting of all the treatment combinations constituting the full factorial was extracted as described in Section 3, B.1.1.1 at each time period (0, 1, 3, 7, 14, and 28 days), and the amount of VX present in each extract was measured by gas chromatography (GC). The analysis was performed as described in Section 3, B.2.2.

(2) GB on soil

Only a summary of the GB soil closed-flask results will be presented here. The full publication of results can be found elsewhere (reference 2). These experiments were run as 4x3x3 factorials, with temperatures of 10, 20, 30 and 40 ^{o}C, soil moisture contents of 1, 11 and 21 percent, and initial agent concentrations of 10, 100 and 1,000 μg GB/g soil.

B. Open-Container Experiments

(1) <u>VX on Soil</u>

This study was conducted in an environmental chamber as a 2x2x2x2 factorial experiment. In addition to the factors investigated in the closed-flask experiments, wind speed was included. The levels for each factor were: soil moistures of 1.2 and 4.3 percent (these percentages resulted from equilibrating the soil in the environmental chamber at 2.5 or 98 percent relative humidity); wind speeds of 0 and 4 mph (6.4 kph); temperatures of 10 and 40 °C; and initial contamination levels of 100 and 1,000 µg VX/g of the test area soil. Multiple samples were prepared of each treatment combination so that a set could be extracted periodically to allow determining VX half-life. The samples were extracted as described in Section 3, B.1.1.1. The VX content of the samples was measured using the GC procedures described in Section 3, B.2.2. The decomposition products were identified using thin layer chromatography as described in Section 3, B.2.3.

(2) <u>GB on Soil</u>

These experiments were run as 2x2x2x2 factorial experiments in an environmental chamber under conditions that yielded 1.2 and 4.3 percent water in the soil, at temperatures of 10 and 40 °C, with agent concentrations of 100 and 1000 µg GB/g soil, and at wind speeds of 0 and 4 mph. Multiple samples were prepared of each treatment combination so a set could be extracted periodically to allow determining GB half-life. The samples were extracted as described in Section 3, B.1.1.2.

2.1.2 SURFACE WATER INVESTIGATIONS

2.1.2.1 <u>Water Studied</u>

Surface waters were collected from two sources at or near DPG. The sources were the run-off pond at Whiterock and brackish ponds dug into the water-table north of Baker Area. A partial chemical analysis of the two water samples is shown in Table 13, Section 2, 2.2.2.

2.1.2.2 <u>Sample Preparation</u>

A. VX Experiments

Two portions (one for each temperature) of each water sample were placed in large stoppered flasks and contaminated with sufficient undiluted VX to produce an agent concentration of 100 μg VX/mL water. The samples were placed in a water bath set at the desired temperature. This was repeated with two additional portions in which the agent concentration was 1,000 μg VX/mL water.

B. GB Experiments

The samples were collected and prepared in a similar manner as described above for VX.

2.1.2.3 Experiment Design

A. VX in Water

The effect of water temperature and agent concentration upon VX decay was determined for each of the two water samples using 2x2 factorial experiments. The levels used were: water temperature, 10 and 40 ^{o}C; and initial agent concentration, 100 and 1,000 μg VX/mL water. Aliquots (1 mL) were withdrawn immediately to establish the initial VX concentration and again at appropriately spaced intervals to determine the agent half-life at each of the combinations of temperature and VX concentration. The aliquots were diluted with 9 mL of 2-propanol, thoroughly mixed, and analyzed for residual VX using the GC procedures described in Section 3, B.2.2. The samples that were taken after the agent decay had passed through one half-life were also propylated and analyzed for decomposition products using the procedures described in Section 3, B.2.1.1, and B.2.2.

B. GB in Water

The decay of GB in surface ponds was determined by 4x2 factorials using water temperatures of 10, 20, 30, and 40 ^{o}C, and initial GB concentrations of 10 and 100 μg GB/mL water. Samples were withdrawn at intervals and analyzed so that half-lifes could be determined. The analysis was conducted as described in Section 3, B.2.1.2.

2.1.3 VEGETATION INVESTIGATIONS

2.1.3.1 Kinds of Vegetation Studied

The vegetation investigation was divided into three sections; dead vegetation, freshly harvested living vegetation, and actively growing vegetation. Except for bean plants, the plant species used are indigenous to the DPG area. The plant species that were used in the dead vegetation section were: cheatgrass (Bromus tectorum, L), budsage (Artemisia spinescens, D.C. Eat.), greasewood (Sarcobatus vermiculatus, (Hook.) Torr.), and the bark from the juniper tree (Juniperus osteosperma, (Torr.) Little). The living vegetation samples were taken from growing or dormant plants found in the DPG test area and include: greasewood (Sarcobatus vermiculatus, (Hook.) Torr.), winter fat (Eurotia lanatus, (Pursh.) Moq.), and sagebrush (Artemisia tridentata, Nutt.).

Considerable time was devoted to selecting the actively growing plant species to be used in this study. Difficulty was experienced in cultivating many of the endemic plant species in the laboratory. Therefore, the majority of the plant studies were conducted using pinto beans (Phaseolis vulgaris, var. humilis), which are easy to cultivate in vermiculite test beds or in trays containing nutrient solutions. However, the need to establish the fate of agents in plants endemic to DPG persisted. As a result, limited laboratory investigations were conducted with budsage and cheat grass. These two endemic species were purposely selected because of their wide differences in morphology and taxonomy.

Budsage was selected as one of the plant species to be studied because it is a shallow-rooted shrub ten to twelve inches tall, that is relatively easy to transplant from the desert to the laboratory. Budsage belongs to the Compositae or dwarf shrub family. This plant is a dicotyledon and has many small leaves on each branch. The leaf veins are netted. The stem and branches are protected by bark and the plant is a perennial. It is commonly found in the piedmonts and foothills but also can be found in the clay flats. This plant is reputed to be especially palatable to grazing wildlife and livestock and is widely distributed throughout the Great Salt Lake Desert.

Cheat grass is an annual that is scattered throughout the uplands of DPG and extends down into the test areas. It is

mixed in with the other plants found in many of the shrub communities. Cheat grass can be found in pure stands on the piedmont areas of Camelback and Granite Mountains which are located in the middle of the test area. It is also frequently scattered with the natural vegetation at the base of shrubs, particularly sagebrush, throughout the test area. Being a drought evader, it has a very short growing cycle in which it grows, flowers, forms seeds, and dies early in the summer. Cheat grass is a member of Gramineae or grass family. The plant is a monocotyledon. The leaves are long and narrow and 3 or 4 are spaced along the lower portion of the single short stem. The leaf veins are numerous and parallel.

2.1.3.2 Sample Preparation

A. Dead Plant Materials

(1) VX Experiments

A large portion of dry cheat grass was ground in a Wiley mill and dried overnight in a vacuum oven set at 60 °C. Two large portions of the dried grass were weighed and placed in larger Erlenmeyer flasks. The moisture content of the grass was adjusted to 1 and 11 percent moisture by adding distilled water. A drop of toluene was added to each flask to retard mold growth. The samples were stopper, mixed, and allowed to equilibrate overnight. From each flask of moisture-adjusted vegetation, two sets of samples were prepared by weighing 3 grams of grass into 125 mL Erlenmeyer flasks. VX dissolved in 2-propanol was added to the grass as small droplets to produce concentrations of 100 and 1,000 μg VX/g (using the same procedure as prescribed for soil). The flasks were stoppered, thoroughly mixed, and stored at the desired temperature. Only closed-flask studies were conducted because the grass blew out of the petri dishes used in the wind tunnel contained in the environmental chamber.

Samples of dead greasewood, budsage, and juniper bark were also collected and samples of each were prepared as described for cheat grass.

(2) GB Experiments

Three batches of dried cheat grass were prepared in the manner described for VX and extensively discussed elsewhere

(reference 2). A summary will be presented here. Water was added to make the samples 1, 11, and 21 percent moisture. These were divided into samples and contaminated with GB that was dissolved in 2-propanol to yield concentrations of 100 and 1,000 μg/g.

B. Freshly Harvested Plants (VX Only)

Samples of growing greasewood and dormant winter samples of winter fat and sagebrush were collected in the DPG test area and returned to the laboratory for preparation. The samples were either ground in a Wiley mill or cut into small pieces with a razor blade. From each sample 1 gram portions were weighed into 125 mL Erlenmeyer flasks. VX dissolved in 2-propanol was added to the plant samples as small droplets to produce the desired concentration of agent/g of plant material. The flasks were stopped, thoroughly mixed, and stored at room temperature.

C. Actively Growing Plants

(1) <u>VX Experiments</u>

The bean plants were raised hydroponically in an environmental inclosure, with the temperature maintained at 20 to 25 ^{o}C. A combination of fluorescent and incandescent lights was used and the intensity of light was approximately 400 foot candles. The illumination cycle was 12 hours light and 12 hours dark. Seedlings were grown in stainless steel trays containing the nutrient solution and the actively growing mature plants were contained in individual Erlenmeyer flasks and were aerated. Plants used in the topical application studies were raised in growth beds containing vermiculite and were watered with nutrient solutions. The nutrient solution used in these studies was Hoagland Number 2 (reference 10).

At the end of each growth period, plants were harvested, sectioned, weighed, and then extracted. Seedlings were sectioned into roots, hypocotyl, and cotyledons (including cotyledonary leaves). The more mature plants (actively growing) used for uptake studies were sectioned into roots, stems, primary leaves, and all other leaves. Plants used for the topical application studies were sectioned similarly to those in the actively growing plant uptake studies except that the contaminated leaf was treated and extracted separately from the

other leaves. The plants used for stem topical application studies were sectioned as previously described except the stem was divided into the section above and below the point of application.

Each time a plant that was exposed to VX was harvested, a control plant (a plant grown in uncontaminated nutrient or a plant not topically contaminated) was harvested for comparative purposes.

The VX plant studies were conducted only with bean plants.

(2) GB Experiments

The bean plant experiments conducted with GB were performed as described for VX and have been discussed elsewhere (reference 2). Only a summary of the results will be provided in this report.

Budsage plants (approximately three years old) were brought to the laboratory during the early winter. The three year old budsage is a small bush (10 to 12 inches high) and has a shallow root system. Because of the small size of this plant, a soil ball containing the plant was dug up leaving the roots undisturbed. The plants were placed in large planters in the laboratory. They were placed under artificial light in the environmental enclosure and watered on a daily basis with distilled water. Within 7 to 10 days sprouts were observed. The plants were allowed to grow in this manner for 2 to 3 additional weeks until heavy leafing was observed on each branch of the budsage bush. At this point, the soil was carefully washed away from the roots with a gentle stream of water and the individual plants were transferred to 1-liter beakers containing a soil nutrient solution. Aeration of the solution was started.

Attempts were made to raise the desert plants in the Hoagland solution. This nutrient was very carefully formulated and can be used to cultivate most plants hydroponically. These attempts were unsuccessful because the pH of the nutrient is 5.5. The desert plants are accustomed to a much higher pH environment. When the pH of the Hoagland solution was raised to 7.5 to 8, (the pH of soil solutions prepared from soil collected from each area) precipitation of some of the minerals in the solution occurred. As a result, soil nutrient solutions were used. The soil nutrient solution was prepared from soil

collected from the budsage area of DPG by mixing the soil for one hour with 1,000 mL of distilled water. The solution was filtered, sterilized, and used as needed.

Cheat grass germinates in the fall and the small plant overwinters and grows to maturity in the early to mid spring. The cheat grass for the experiment was collected in the spring. This was accomplished by transferring clumps of soil containing cheat grass to the laboratory environmental enclosure and watering them until fully mature cheat grass plants were obtained. The plants were then carefully separated from the soil clumps and transferred to 125 mL Erlenmyer flasks containing a soil nutrient solution prepared as described before, using soil collected from a cheat grass area of DPG (piedmont foothill area around around the test areas). Because of the small size of each individual cheat grass plant, 3 plants were placed in each flask.

2.1.3.3 Experiment Design

A. Dead Plant Materials

(1) VX Experiments

A 3x2x2 factorial experiment was used to study the effect of temperature, moisture level, and agent concentration upon the decay of VX in dead cheatgrass. The levels used were: temperatures of 10, 25, and 40 °C; vegetation moisture contents of 1 and 11 percent; and initial agent concentrations of 100 and 1,000 µg VX/g vegetation. Samples were extracted at 0, 1, 3, 7, 14, 28 and 56 days using the procedures described in Section 3, B.1.2.1. The extracts were analyzed for residual VX using the methods described in Section 3, B.2.2.

Samples of dead greasewood, budsage, and juniper bark were contaminated with VX in order to compare the agent persistence in a variety of plant species in addition to cheat grass indigenous to DPG. The moisture content of the samples were "as received" from the field and were as follows: greasewood 6.8 percent, budsage 4.4 percent, and juniper bark 6.4 percent. The samples were stored at room temperature (20-25 °C). The initial agent concentration were 100 and 1,000 µg VX/g vegetation. The samples were extracted at 0, 1, 3, 5, 7, 10, 15, 20, 25, and 50 days using the procedures described in Section 3, B.1.2.1, extracts were analyzed as described in Section 3, B.2.2. The presence of decomposition products was detected as described in Section 3, B.2.3.

(2) GB Experiments

A 4x3x3 factorial experiment was used to study the effect of temperature, moisture level, and agent concentration upon the decay of GB in dead cheat grass. The levels used were: temperature: 10, 20, 30, and 40 °C; moisture level: 1, 11, and 21 percent; initial agent concentration: 10, 100, and 1,000 μg GB/g vegetation. Samples were extracted at 0, 6, and 12 hours; 1, 2, and 4 days using the procedure described in Section 3, B.1.2.2. The extracts were analyzed using the GC method described in Section 3, B.2.2.

B. Freshly Harvested Plants (VX Only)

Samples of living vegetation were contaminated with VX in order to compare the agent persistence in living and dead vegetation. Living but dormant specimens of sagebrush and winter fat and budding greasewood samples were contaminated at 100 and 1,000 μg VX/g vegetation levels. The samples were thoroughly mixed, stoppered, and stored at room temperature. Samples were extracted at 0, 1, 3, 5, and 7 days using the procedures described in Section 3, B.1.2.1. The extracts were analyzed as described in Section 3, B.2.2.

C. Actively Growing Plants

(1) VX Experiments

Investigations into the uptake, translocation, and decay of VX in actively growing vegetation were conducted using bean plants only.

A series of studies on the uptake of VX by seed was conducted by placing bean seeds in contact with VX-treated nutrient solution. The contamination level was 43 μg VX/mL solution. The solution containing VX and a control consisting of nutrient solution without VX were transferred into 2 stainless steel trays, each of which contained a wire supporting rack. These racks permitted the bean seeds to just touch the surface of the liquid. The trays were covered and the nutrient was maintained at a constant level to assure that the liquid would remain in contact with the seeds. During the germination period, the seeds and sprouts were kept in the dark (etiolated). The nutrient was sampled periodically and analyzed for VX. Bean seeds were removed at 1, 4, 7, and 11 days. Each

seed or seedling of the series was rinsed and extracted by maceration in a tissue homogenizer using the procedure described in Section 3, B.1.2.1. The extract was analyzed for residual VX using the procedure described in Section 3, B.2.2. Also the presence of VX decomposition products was determined using the procedures given in Section 3, B.2.2 and B.2.2.5.

At day 7 and 11, a number of seedlings were transferred to uncontaminated nutrient. The plants were placed under artificial light and aeration of the roots was initiated. The plants that were transferred at 7 days were allowed to grow for 17 additional days, while those transferred at 11 days grew for 13 additional days. At the end of these times, plants from each set were harvested, and separated into roots, stem, and leaf fractions. Each fraction was extracted and the extract analyzed for VX using the procedures described in Section 3, B.2.2, and B.1.2.1. Plants from each set were also harvested at 33 and 37 days respectively, separated and extracted as before, and the extract analyzed.

Another series of experiments was conducted to study the uptake of high concentrations of VX from nutrient solutions. The concentrations used were 250, 500 and 1,000 micrograms VX/mL nutrient solution. Aliquots of each solution were transferred to stainless steel trays (nutrient solution without VX was used as a control), and bean seeds were placed upon a wire mesh rack in the nutrient solution so that each bean seed just touched the surface of the nutrient solution. The level of nutrient in each tray was maintained over the course of the experiment. Seeds were harvested at 1, 7, and 9 days, rinsed and extracted, and analyzed. At day 9 the remaining seeds were exposed to artificial light for 12-hour periods, followed by 12 hours of darkness. The roots were aerated (plants that are hydroponically grown are more vigorous if the roots are aerated). At 18 days all plants were transferred to uncontaminated nutrient solution contained in flasks and exposed to artificial light for 12-hour periods followed by 12 hours of darkness. Aeration of the roots was continued. Plants were harvested at 4, 11, 18, and 25 days after their transfer to uncontaminated nutrient solution. The plants were sectioned into primary leaves, secondary leaves, upper stem, lower stem, and root sections. Each section was extracted and the extracts analyzed for VX by GC as described in Section 3, B.1.2.1 and B.2.2.

The fate of agent topically applied to plants was also studied. Beans were planted in a vermiculite growth bed and watered with Hoagland Solution. After the development of primary leaves, 1 microliter of VX was applied to selected

plants at one of the following locations: (a) apical meristem, (b) stem just above the dicotyledon casings, and c) at the apex of one of the primary leaves. At the time of application, one plant from each set was harvested, and sectioned into roots, stems, and leaf fractions. Each fraction was extracted using the procedure described in Section 3, B.1.2.1 and the extracts analyzed for VX by GC to establish zero-time levels. Additional plants were harvested at 3, 9, and 22 days after the application of VX. The resulting extracts were analyzed by GC. The analysis procedure is described in Section 3, B.2.2. The presence of VX related decomposition products in the plant extracts was detected and measured using the methods listed in Section 3, B.1.3, B.2.2, B.2.2.5, and B.2.3.

Bean plants were sprouted in a nutrient media to study uptake, translocation, and disappearance of the decomposition products YN, YL, and IMP. Subsequent analysis of the YL used indicated that disproportionation had occurred during preparation, resulting in the material used for application being predominantly WJ and YN. The samples taken at sprouting, mid-vegetative, flowering, and fruit formation phases of plant growth, and analyzed for YL, were then reanalyzed for WJ. The methods used for extraction and analysis of the decomposition products are given in Section 3, B.1.3, B.2.2, and B.2.2.5.

(2) GB Experiments

The uptake, translocation, and persistence of GB in bean plants has been extensively studied and reported elsewhere (references 2 and 3). Both isotopically labeled and unlabeled GB were used in this investigation. The distribution of the isotope throughout the plant and the chemical identity of the label was determined using the procedures listed in Section 3, B.1.2.2, B.1.3, B.2.2, B.2.2.5, B.2.4, and B.2.5.

Both isotopically labeled and unlabeled GB were also used in different portions of the studies to study the uptake, translocation, and accumulation of the agent in budsage and cheat grass. In addition, the appearance, accumulation, and elimination of IMP, a hydrolytic decomposition product of GB, was studied using budsage during these experiments. In the plant uptake studies, the concentration of GB in the nutrient ranged from 10 to 1,000 μg/mL. In the topical application studies, one microliter of agent was placed either on a primary leaf or on the stem below the primary leaves. The extraction and analysis of the samples were conducted using the methods listed in Section 3, B.1.2.2, B.2.2, and B.2.2.5.

2.1.4 ANALYSIS METHODS AND TECHNIQUES

Analytical methods used to detect and quantitatively measure the agents or associated decomposition products in the test samples are too numerous and extensive to list here. Each method is discussed in Section 3, Appendix B of this report. The extraction procedures used in this investigation are also presented in Section 3, Appendix B.

2.1.5 EQUIPMENT

2.1.5.1 Gas Chromatograph (GC)

All extracts were analyzed quantitatively for GB and VX content using a Varian-Aerograph Model 1520B or a Varian-Aerograph Model 1800 gas chromatograph equipped with Melpar Flame Photometric Detectors (Model FPD 200) marketed by Tracor, Inc., Austin, Texas.

Extracts propylated to determine amounts of decomposition products were also analyzed by gas chromatography using propylated standards of IMP and YN. The operational parameters and columns used are listed in Section 3, B.2.2.

2.1.5.2 Radiological Equipment

The soil and dry vegetation samples containing radioisotope labeled agents and extracts of these samples were counted with a Nuclear Chicago Ultrascaler Model 192A equipped with a Geiger-Mueller tube, Type D34, with an end window diameter of 1-3/8 in. and thickness of 1.4 mg/cm. The total radioactivity in plant extracts was also measured using a Nuclear-Chicago Mark II liquid scintillation counter. The operation and experimental parameters are listed in Section 3, B.2.4 and B.2.5.

2.1.6 CONTROLS AND AGENT PURITY

Fresh agent standards were prepared in 2-propanol each week. This was done to insure the reliability of the analysis and to determine agent stability in 2-propanol. The agent

standards were analyzed by GC daily. If the instrumental response decreased by more than 10 percent, fresh standards were prepared. The VX and GB samples used in these experiments were stable in 2-propanol for 3 to 4 weeks.

Controls for the soil investigations were prepared by extracting moisture adjusted soil samples from each soil area with 2-propanol. These extracts were then used to prepare agent standards which were analyzed daily. These standards were used to quantitate the sample extracts and were compared to the 2-propanol standards to determine the stability of VX and GB in soil extracts.

All water samples containing agent were compared to water control standards prepared in a solution containing 1 part water and 9 parts 2-propanol. The water control standards were analyzed daily and compared to standards of agent prepared in 2-propanol.

Vegetation controls were prepared by extracting an appropriate quantity of moisture-adjusted vegetation with 2-propanol or acidified ethanol. Agent standards were prepared in these extracts and the stability was determined by comparing these controls to standards of VX or GB prepared in 2-propanol.

Purity of the VX used in these experiments, determined for each agent sample or lot number, ranged between 90.5 to 94 percent. The unlabeled GB was 93.3 percent pure. The 14C labeled GB was 97.4 percent pure and had a specific activity of 22.5 DPM/μg.

2.1.7 DATA REDUCTION

Data reduction consisted of three major steps: (1) performing an analysis of variance to determine the magnitude of the effect of the experimental factors and their statistical significance, (2) operating on the effects and interactions to obtain a prediction equation, and (3) using the equation to give tabular and graphic predictions over the range of interest for each factor tested.

The first step, analysis of variance, was accomplished by means of Yates' algorithm. For those experiments in which the factors were not all tested at the same number of levels or in which a given factor was tested at four or more levels, Yates' algorithm was modified to apply to these more general cases. The procedure developed parallels that of Yates but adds higher order orthogonal polynomials to extend the utility of the technique.

The second step was derivation of a prediction equation. First, a set of equations were derived to relate the actual value of each level of each factor to the appropriate orthogonal polynomial. Second, these equations were applied to the table of effects and interactions judged to be statistically significant at any given confidence level.

The third step was accomplished by taking the equations and systematically varying each of the factors over the desired ranges, followed by tabulating and plotting the resulting values.

Because factorial experiment designs allow evaluation of interaction, equations derived from such data allow, in principle, more valid extrapolations outside of the actual experimental ranges, if a discontinuity is not encountered in the response surface. The nature of the equations derived requires that every term be included in making extrapolations. Factor levels which cause a term to go to zero, i.e., 0 ^{o}C and 1 μg agent/gm (because log 1 = 0) are not allowed and should be considered as lower limits to temperature and concentration, respectively.

2.2 RESULTS

2.2.1 SOIL INVESTIGATIONS

2.2.1.1 The Decay of VX in Test Area Soil

Shadscale-gray molly-greasewood soil was chosen for this set of VX decay experiments because it is the most prevalent soil in the test area.

A. Closed-Flask Experiments

After the data were determined to follow an exponential decay curve, VX half-lives were calculated for each set of samples run with a given treatment combination. These half-lives were taken as the response to each treatment combination. An analysis of variance on these results showed that the effects of soil moisture, temperature, and agent contamination level each was statistically significant at the 99 percent level compared to the experimental error variance. An equation that incorporate all of the statistically significant

main effects and interactions was derived by least squares regression (the practical significance of a given term probably depends upon the levels assigned to each variable):

$$t_{1/2} = 39.50367 - 2.61787W - 12.11791 \ln C + 0.91079 W \ln C + 1.52922 \ln - 0.08579 W \ln C - 2.02653 T + 0.20619 WT + 0.90643 T \ln C - 0.09521 WT \ln C - 0.10934 T \ln C + 0.01047 WT \ln C + 0.02452 T - 0.00330 WT - 0.01229 T \ln C + 0.00166 WT \ln C + 0.00143 T \ln C - 0.00019 WT \ln C \quad (1)$$

where $t_{1/2}$ = VX half-life (days); W = water content (weight percent); C = VX concentration in soil (μg/g); and T = temperature (^oC).

From this equation it was possible to prepare a table that includes additional levels of each variable so a predicted half-life can be more readily identified for given combinations of these experimental variables (Table 5).

The equation also was used to prepare graphs which are "slices" through the three-dimensional experimental factor space, showing VX half-life in soil in the absence of ventilation over a range of temperatures and moisture contents, at several different initial VX concentrations (Figures 1 to 3).

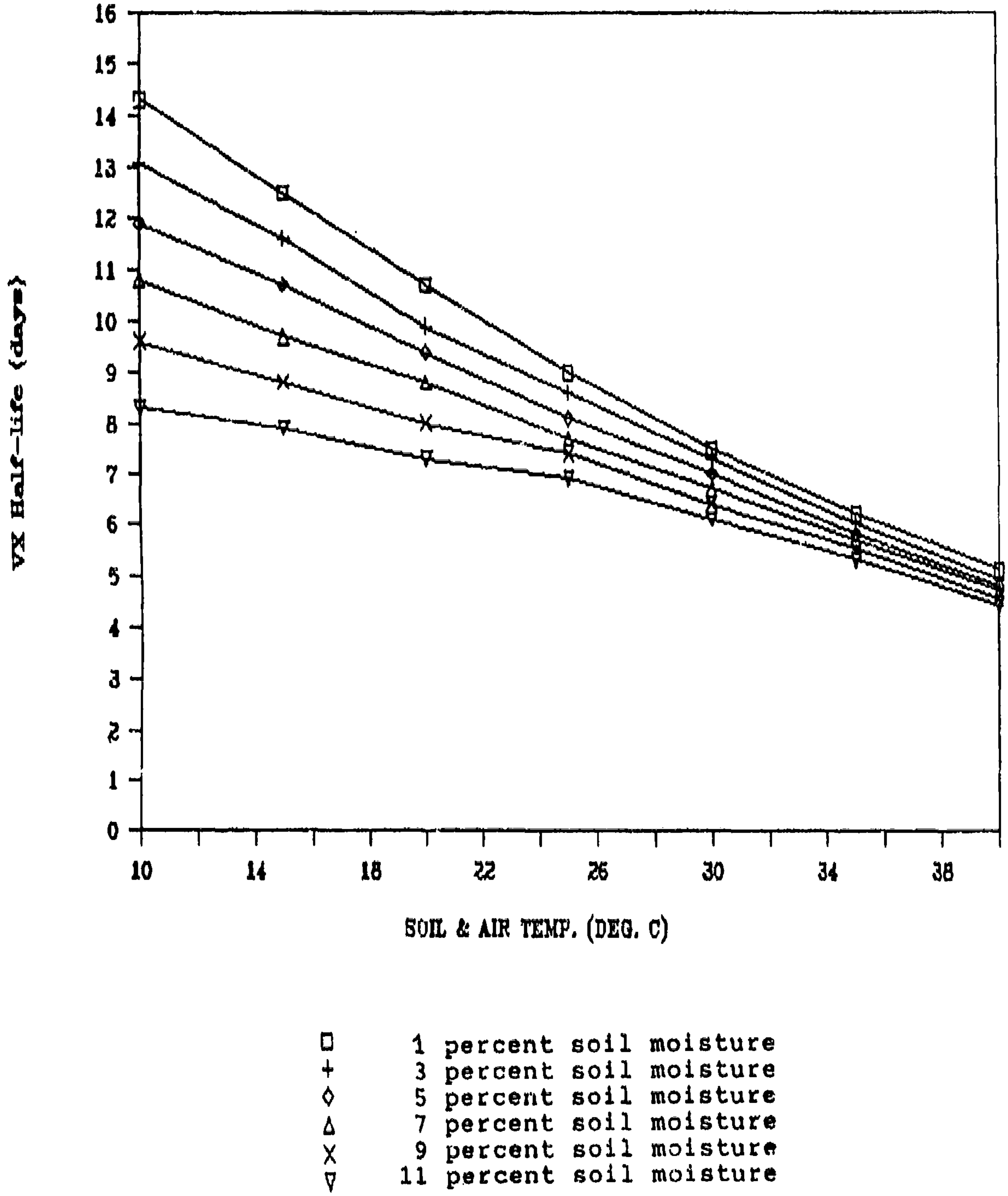


Figure 1. Half Life of VX in Test Area Soil in Closed Flasks, (Initial Concentration was 10 μg VX/g Soil).

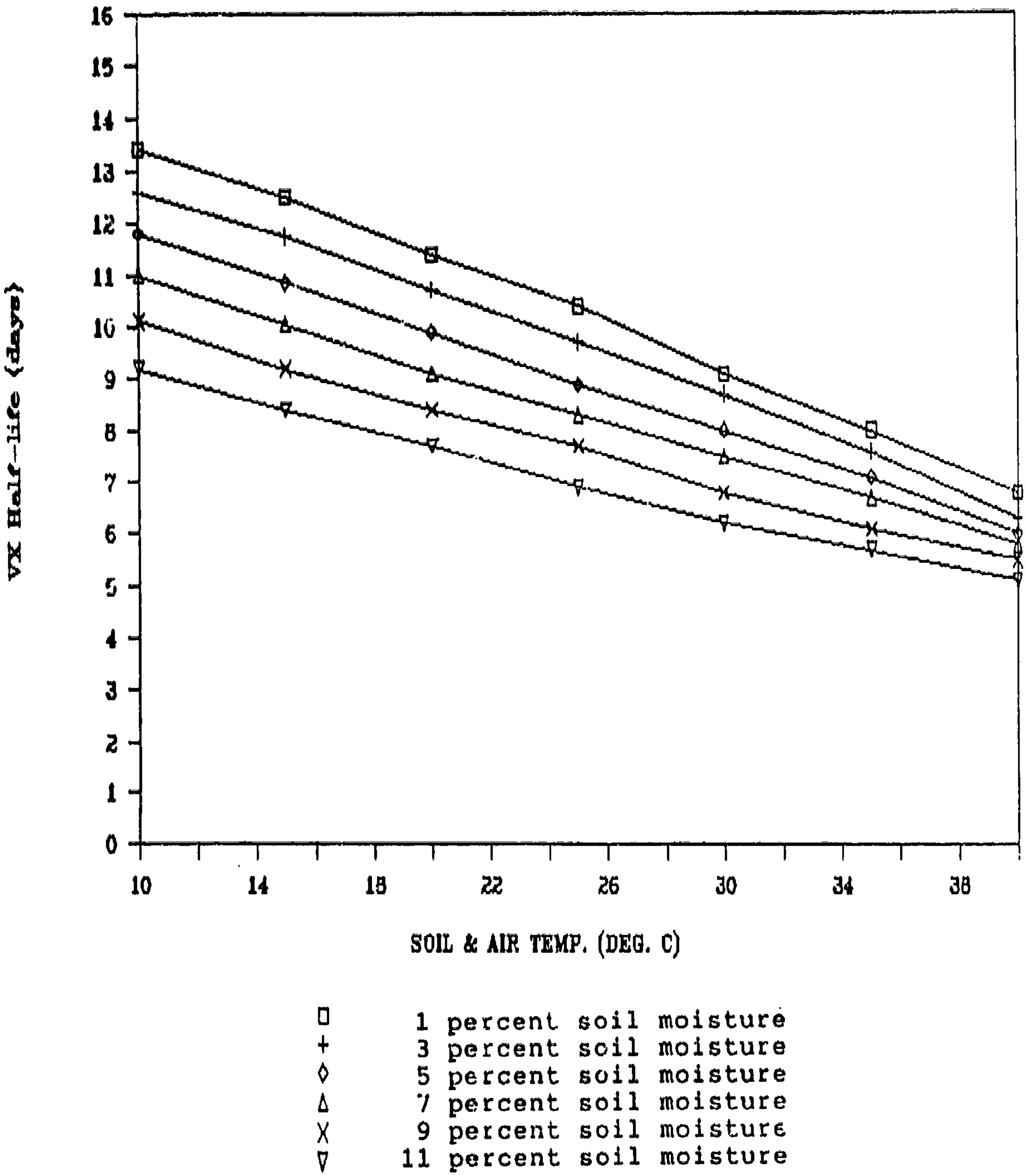


Figure 2. Half-Life of VX in Test Area Soil in Closed Flasks. (Initial Concentration was 50 μg VX/g Soil).

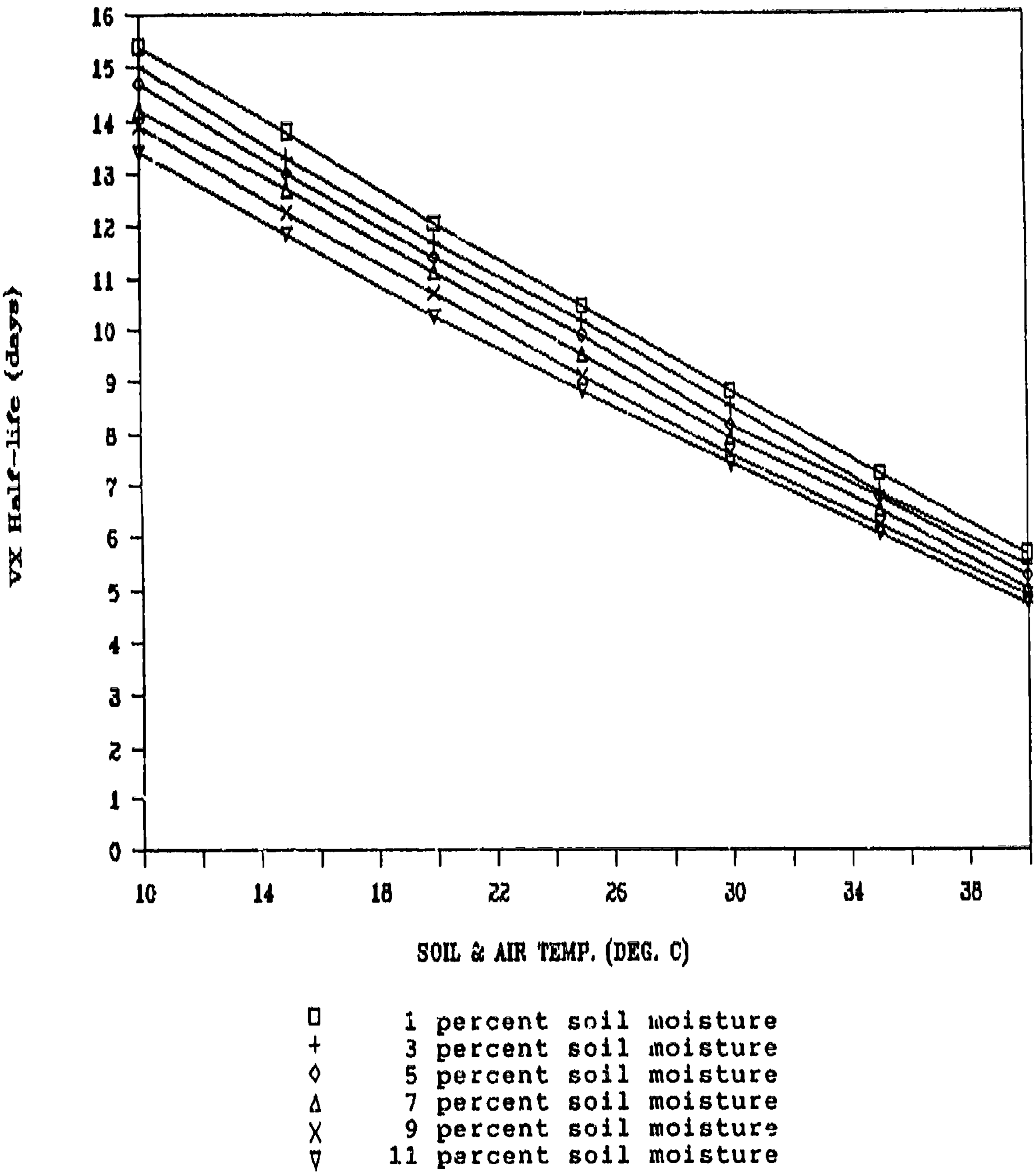


Figure 3. Half-Life of VX in Test Area Soil in Closed Flasks, (Initial Concentration was 250 µg VX/g Soil).

Table 5. Calculated Half-Life (days) of VX on Test Area Soil in Closed Flasks

Temp. (^{o}C)	Initial Conc. ($\mu g/g$)	Moisture Content of Soil (Percent) 1.0	3.0	5.0	7.0	9.0	11.0
10.0	10.0	14.30	13.10	11.90	10.70	9.50	8.30
	22.4	13.48	12.47	11.48	10.48	9.48	8.48
	50.0	13.40	12.60	11.80	11.00	10.20	9.40
	111.8	14.08	13.47	12.88	12.28	11.68	11.08
	250.0	15.50	15.10	14.70	14.30	13.90	13.50
17.5	10.0	11.41	10.65	9.88	9.12	8.35	7.59
	22.4	11.61	10.79	9.97	9.15	8.33	7.51
	50.0	11.92	11.15	10.38	9.61	8.84	8.06
	111.8	12.36	11.74	11.12	10.50	9.87	9.25
	250.0	12.92	12.56	12.18	11.82	11.44	11.08
25.0	10.0	8.90	8.46	8.02	7.58	7.14	6.70
	22.4	9.76	9.13	8.49	7.86	7.22	6.59
	50.0	10.30	9.62	8.94	8.26	7.58	6.90
	111.8	10.51	9.94	9.36	8.79	8.21	7.64
	250.0	10.40	10.08	9.76	9.44	9.12	8.80
32.5	10.0	6.76	6.54	6.31	6.09	5.86	5.64
	22.4	7.94	7.49	7.05	6.60	6.16	5.71
	50.0	8.52	8.00	7.48	6.96	6.44	5.91
	111.8	8.52	8.06	7.60	7.15	6.69	6.23
	250.0	7.92	7.68	7.42	7.18	6.92	6.68
40.0	10.0	5.00	4.88	4.76	4.64	4.52	4.40
	22.4	6.14	5.89	5.64	5.39	5.14	4.89
	50.0	6.60	6.30	6.00	5.70	5.40	5.10
	111.8	6.39	6.12	5.85	5.58	5.31	5.04
	250.0	5.50	5.34	5.18	5.02	4.86	4.70

NOTE: This table is based on data from tests conducted at temperatures of 10, 25 and 40 ^{o}C; soil moisture levels of 1 and 11 percent; initial VX concentrations of 1, 100, and 1,000 μg VX/g soil; and sampling at 0, 3, 7, 14, and 28 days.

Figure 4 summarizes data from this experiment for a starting concentration of 250 μg VX/g soil.

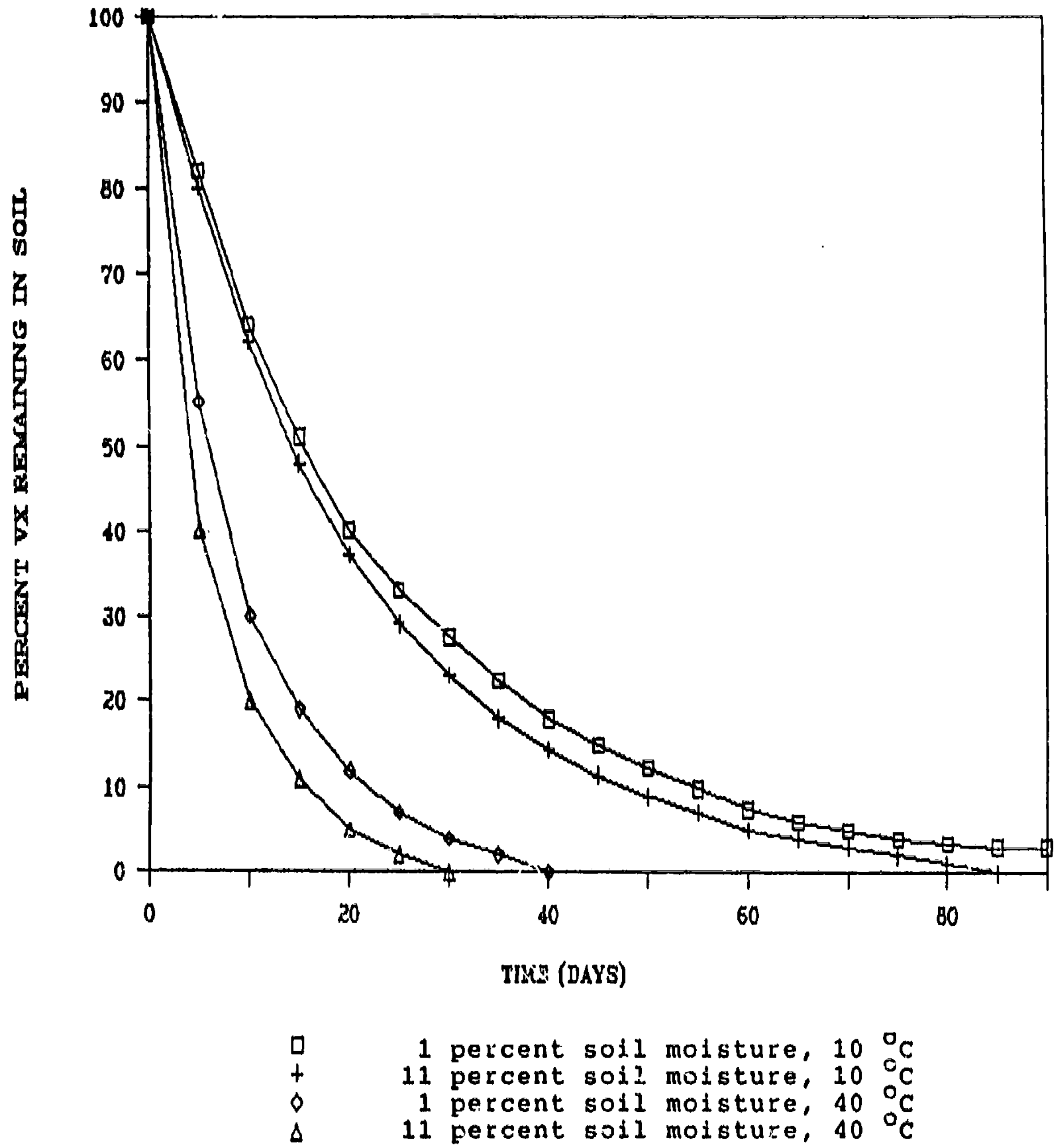


Figure 4. Persistence of VX on Test Area Soil in Closed Flasks. (Initial Concentration = 250 µg/g)

The temperature range used in the above experiments bracket the temperatures normally found at DPG from March through October. It is during this time that most of the field tests are conducted. However, a limited amount of work was conducted at temperatures below freezing (-17 °C). These studies were conducted at several soil moisture levels and the VX contamination level was 10,000 μg/g soil. The flasks were stoppered and not exposed to air circulation. The results are given in Table 6 and shown in Figure 5.

TABLE 6. Recovery of VX From Soil Stored at -17 °C

Time after agent application	Percent VX recovered from soil containing indicated percent moisture			
	0	0.5	1.0	5.0
0.17 days	84	80	79	61
7.0 days	63	54	50	20
14.0 days	62	53	44	33
35.0 days	63	58	41	25

The VX will persist longer at this low temperature than at temperatures between 10 and 40 °C. However, it must be remembered that the samples were not exposed to wind. If they had been, the persistence would have been shorter. Another consideration is that although the temperature at which the samples were stored was below freezing, similar to the air temperatures found during the winter at DPG in the field, the soil surface temperature may be considerably higher than the air temperature in the actual test area during the day. This would accelerate the decomposition of the agent. For example, actual measurements of ground and air temperatures during the winter revealed that during the day when the air temperature was near zero, the ground temperature was 10 °C, and when the air temperature reached 6 °C, the ground was at 20 °C.

B. Open-Container Experiments

The effects were calculated from the difference between the sums of eight experiments at each level. An analysis of

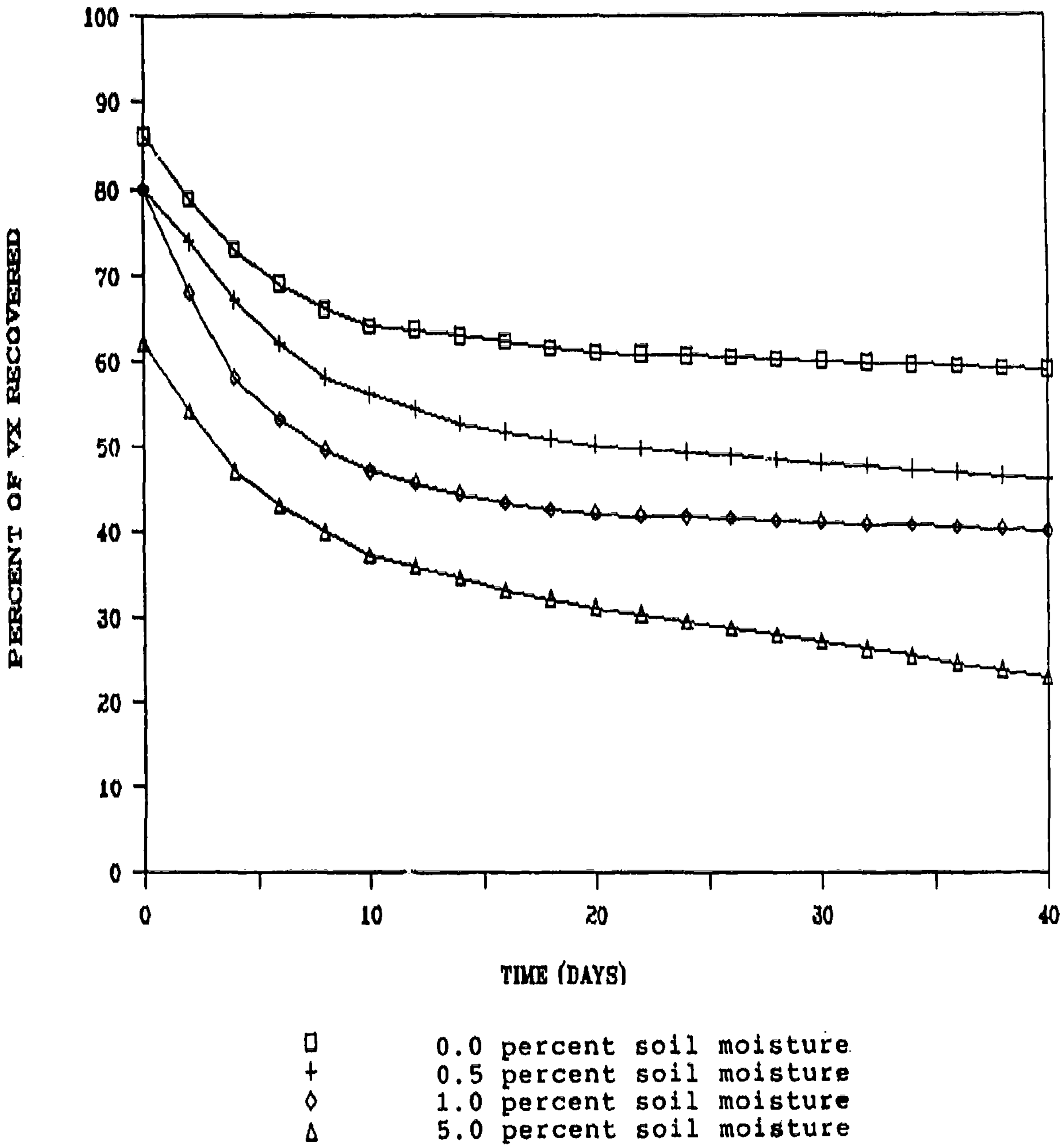


Figure 5. Recovery of VX From Test Area Soil at -17 Deg. C in Closed Flasks.

variance showed that the effect of varying concentration from 100 to 1000 $\mu g/g$ did not significantly affect the half-life; it also revealed that varying the water content of the soil from 1.2 to 4.3 percent was significant only at the 80 percent level, but that temperature and wind speed were significant at the 99 percent level.

Equation 2 was derived from this data and was used to prepare Table 7 and Figures 6 to 9. This equation was also used to prepare the summarizing graph of Figure 10.

$$t_{1/2} = 22.5043 + 0.00016\ C - 0.84506\ V - 0.00002\ CV - 0.73507\ W - 0.00004\ CW - 0.05511\ VW - 0.54237\ T - 0.00002\ CT - 0.01717\ VT + 0.02512\ WT - 0.00148\ VWT \quad (2)$$

where $t_{1/2}$ = half-life (days), C = VX conc ($\mu g/g$), V = wind velocity (mph), W_o = water content (percent), and T = temperature (^oC).

Actual field trials were conducted in the past by the personnel at a different geographic location, using VX for the purpose of determining persistence in grass-covered prairie terrain. VX was applied at a mean contamination density of 4.8 g/m. The field experiment was started in October and continued for one year. The air temperatures for the first six months of the experiment were either at the low temperature used in our chamber studies (10 ^oC) or well below, and the results correlate with our chamber experiments. After eight hours, 90 percent of the VX had vaporized or decomposed. Of the agent remaining on the soil, a portion had decomposed to YL and YN. However, mortalities still were obtained from small Yorkshire white pigs through skin penetration 30 days later (one out of five died), but none thereafter. This persistency would be expected due to the cold temperatures (-16 to +8 ^oC) at the test site during the month of November. A depressed cholinesterase level could be detected in these animals when they were pressed against the ground up to five months after the application of the VX to the test area.

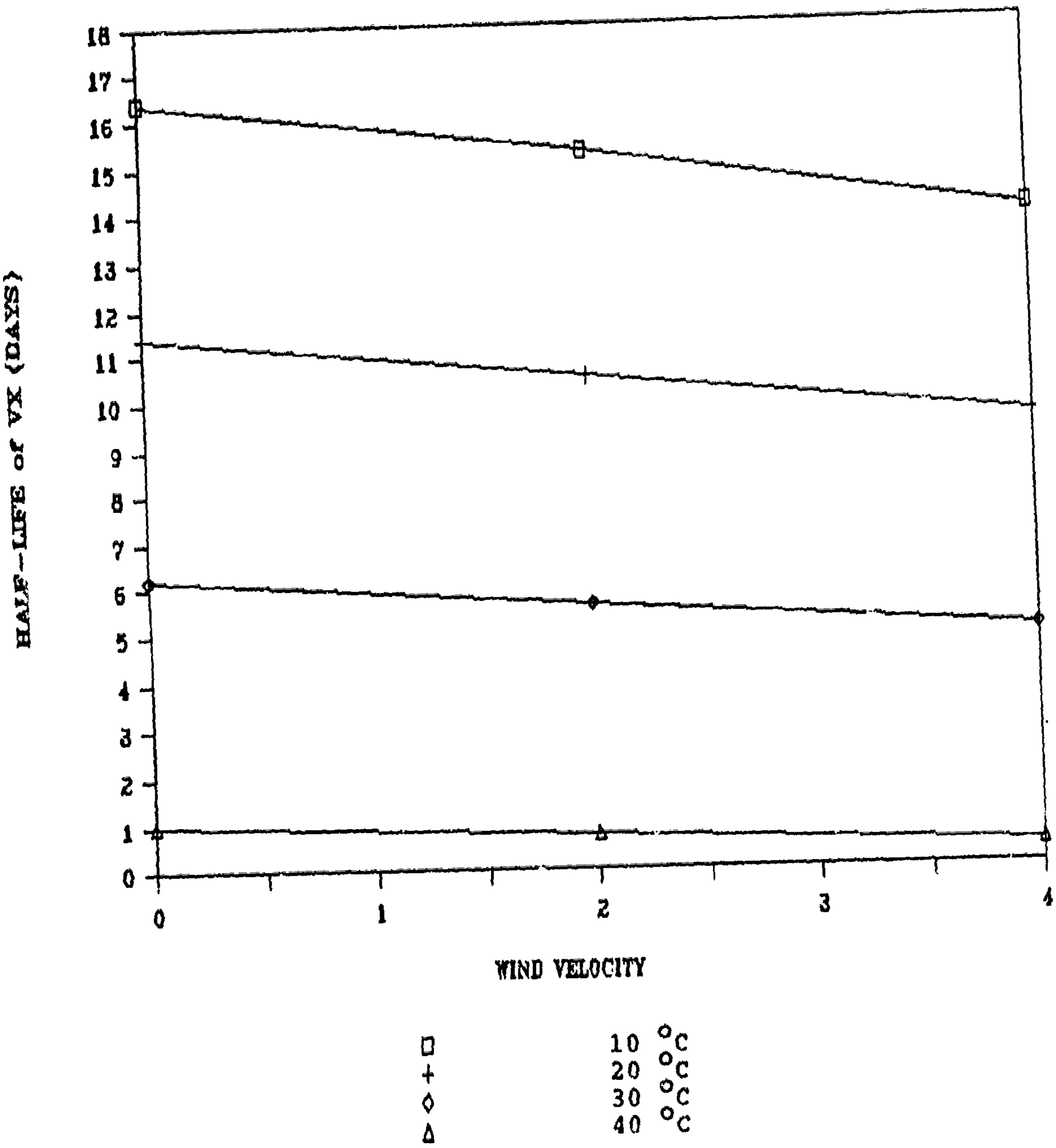


Figure 6. Half-Life of VX on Test Area Soil in Open Containers. (Soil Moisture = 1.2%, Initial Concentration 100 µg VX/g Soil).

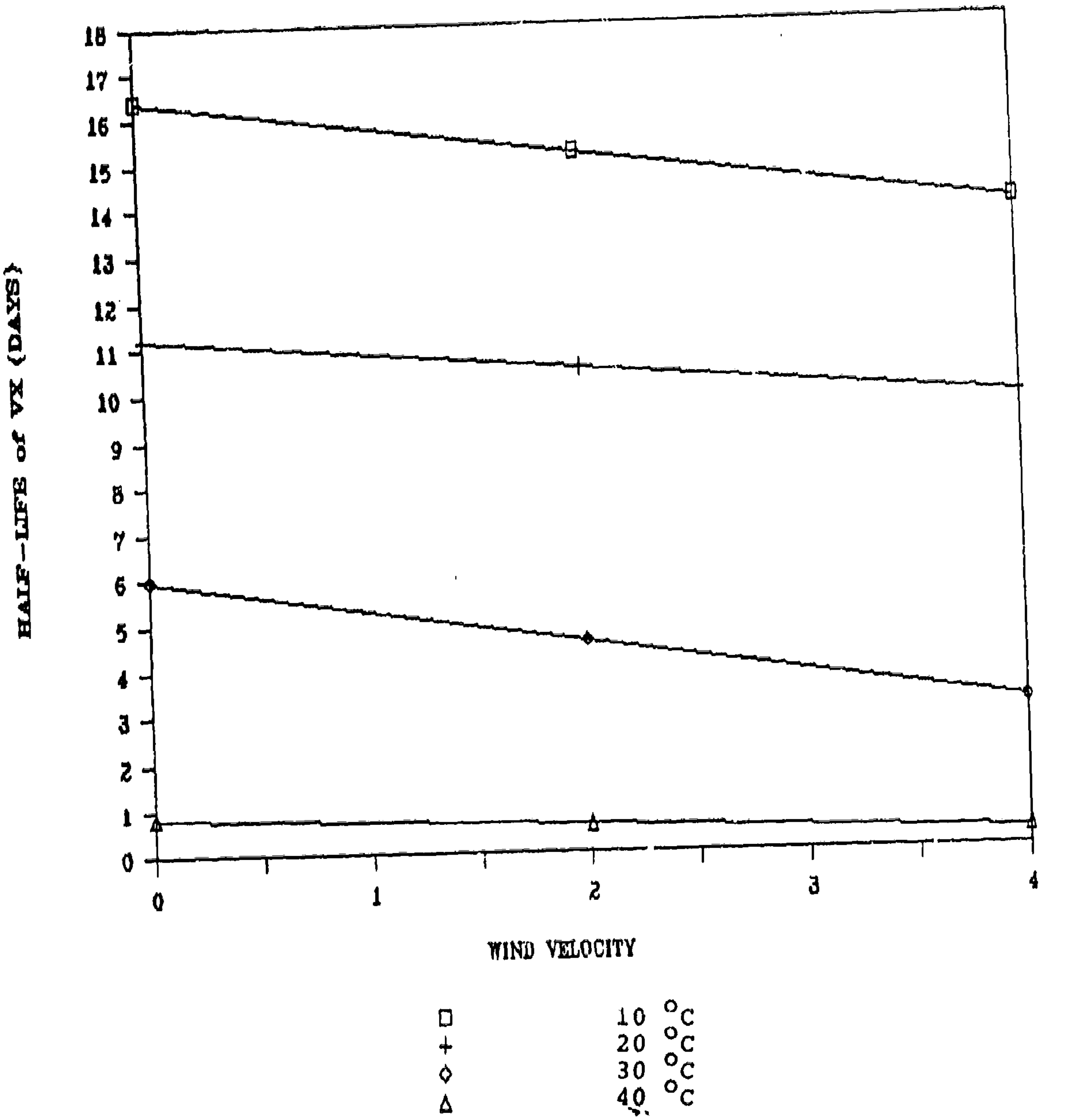


Figure 7. Half-Life of VX on on Test Area Soil in Open Containers. (Soil Moisture = 1.2%. Initial Concentration 1000 µg VX/g Soil).

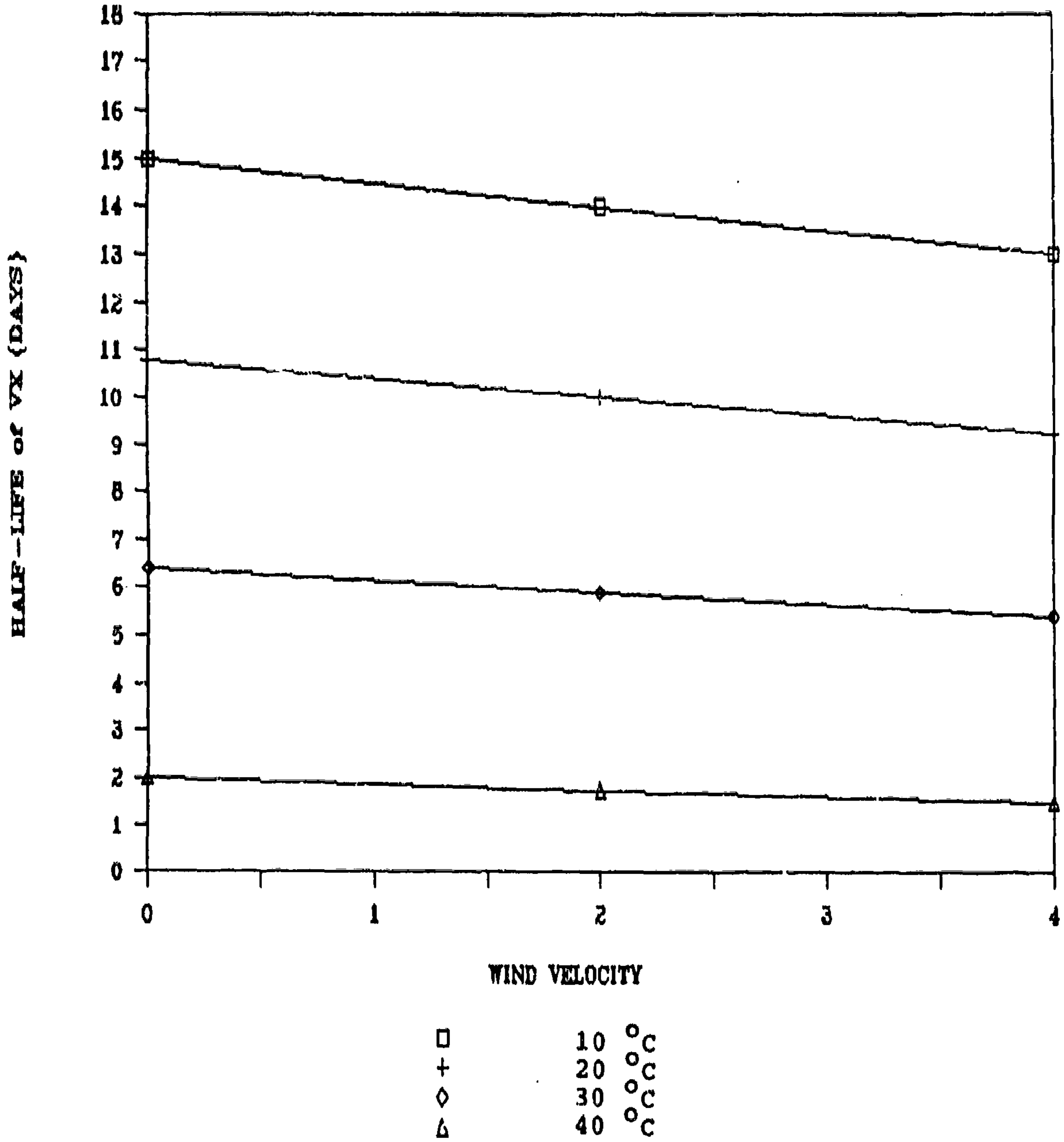


Figure 8. Half-Life of VX on Test Area Soil in Open Containers. (Soil Moisture = 4.3%, Initial Concentration 100 μg VX/g Soil).

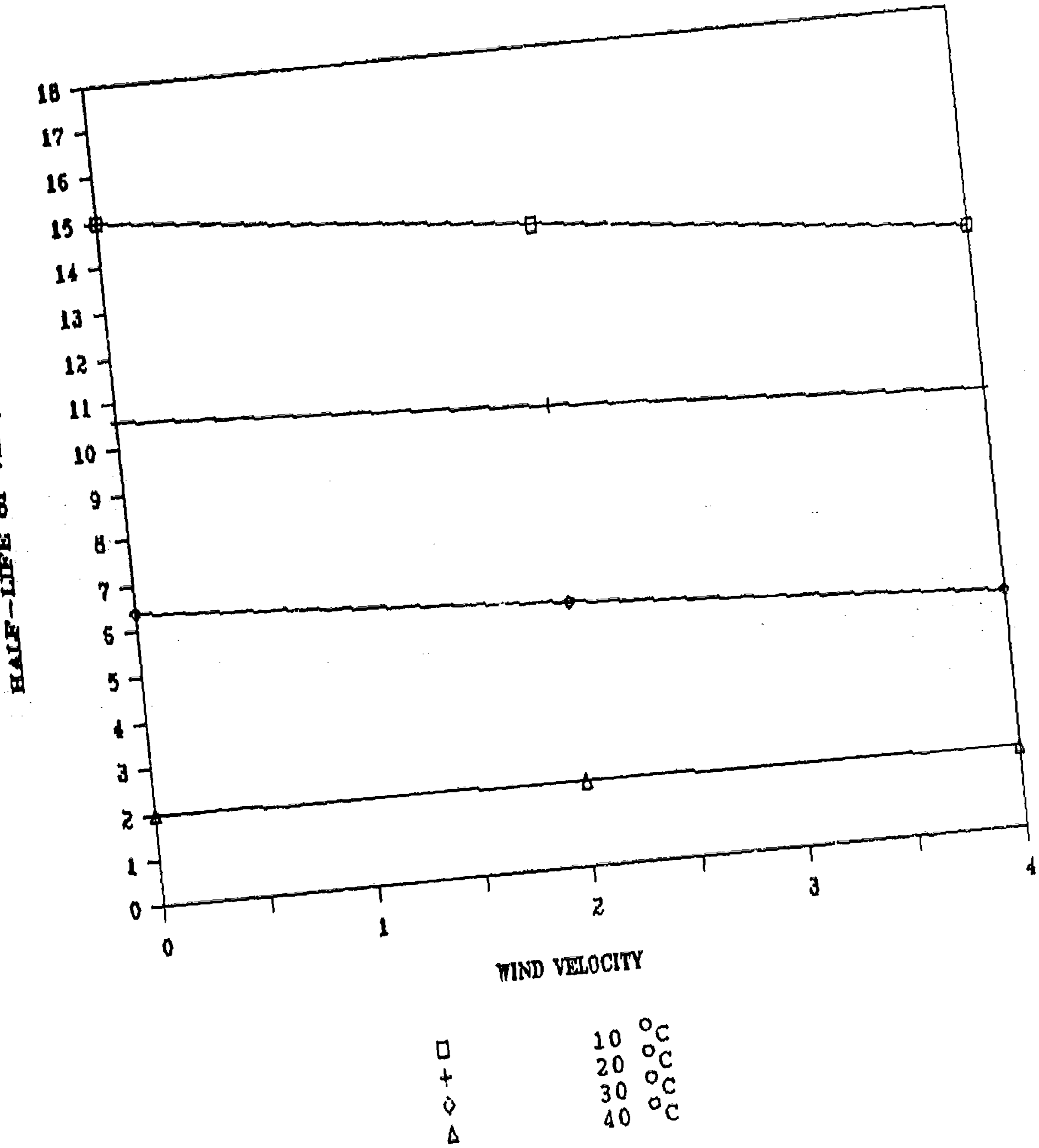


Figure 9. Half-Life of VX on Test Area Soil in Open Containers, (Soil Moisture = 4.3%, Initial Concentration 1000 μg VX/g Soil).

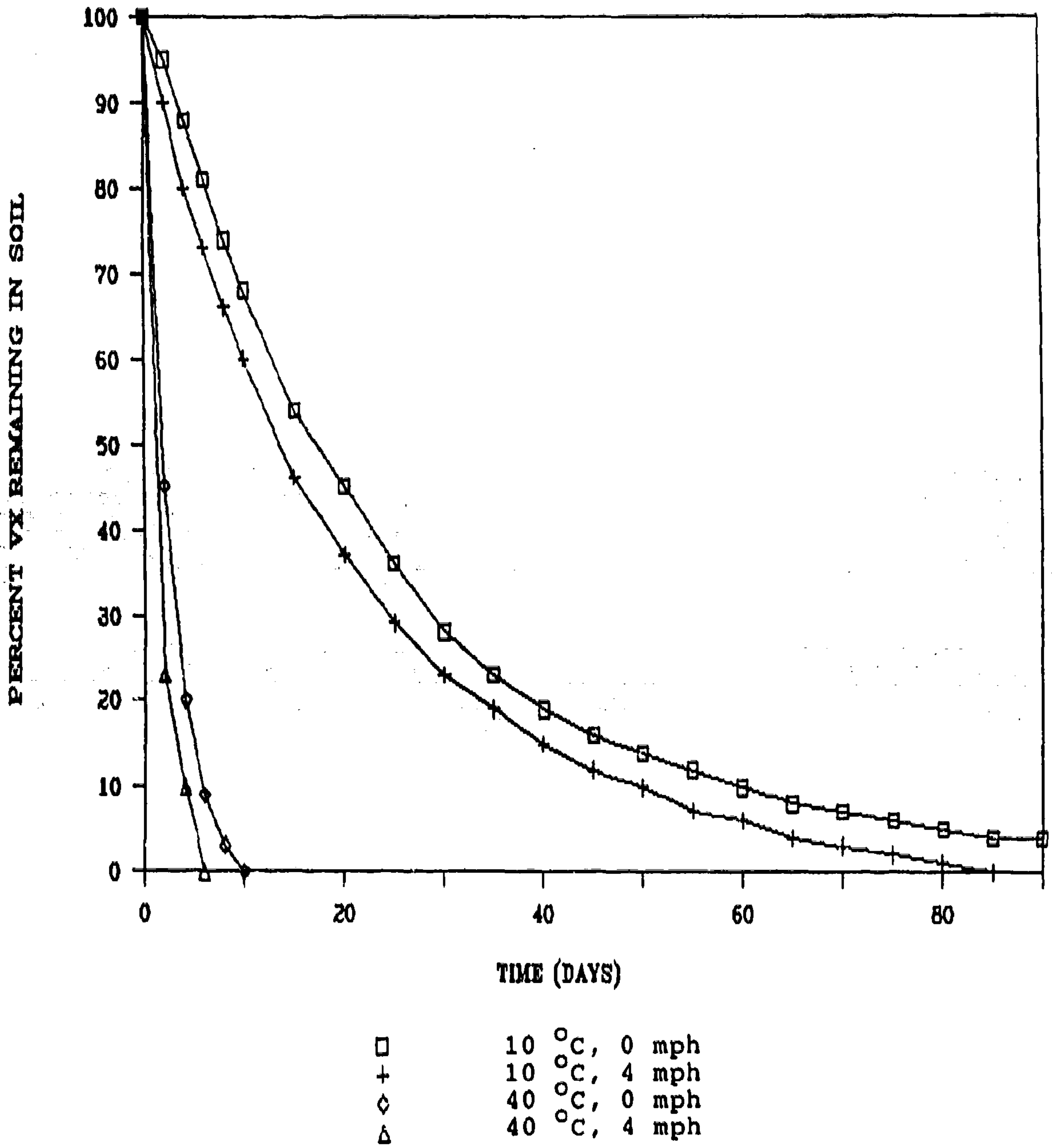


Figure 10. Persistence of VX on Test Area Soil in Open Containers, (Initial Concentration = 100 to 1000 μg/g, Soil Moisture = 1.2 to 4.3% Water).

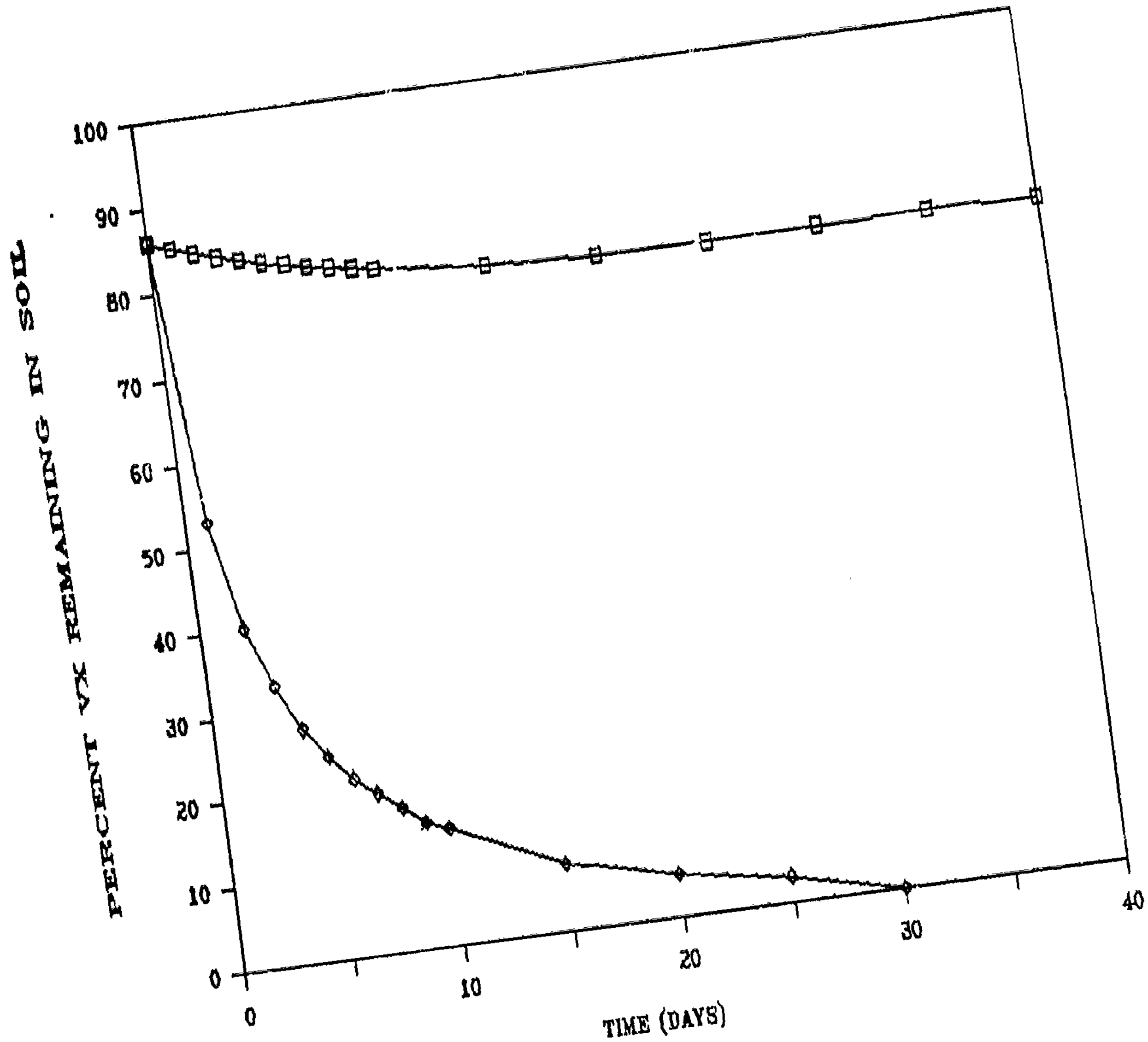
Table 7. Calculated Half-Life (days) of VX on Test Area Soil in Open Containers

Temp. (^{o}C)	Initial Conc. ($\mu g/g$)	Wind Velocity (mph) 0 — Moisture Content of Soil (percent) 1.2	0 — 4.3	4 — 1.2	4 — 4.3
10	100	16.5	15.0	14.0	13.0
	1,000	16.5	15.0	14.0	13.0
20	100	11.4	10.7	9.5	9.1
	1,000	11.3	10.7	9.5	9.2
30	100	6.2	6.3	5.0	5.2
	1,000	6.0	6.4	5.0	5.4
40	100	1.1	2.0	0.5	1.3
	1,000	0.8	2.0	0.4	1.6

C. Mode of Decay of VX in Soil

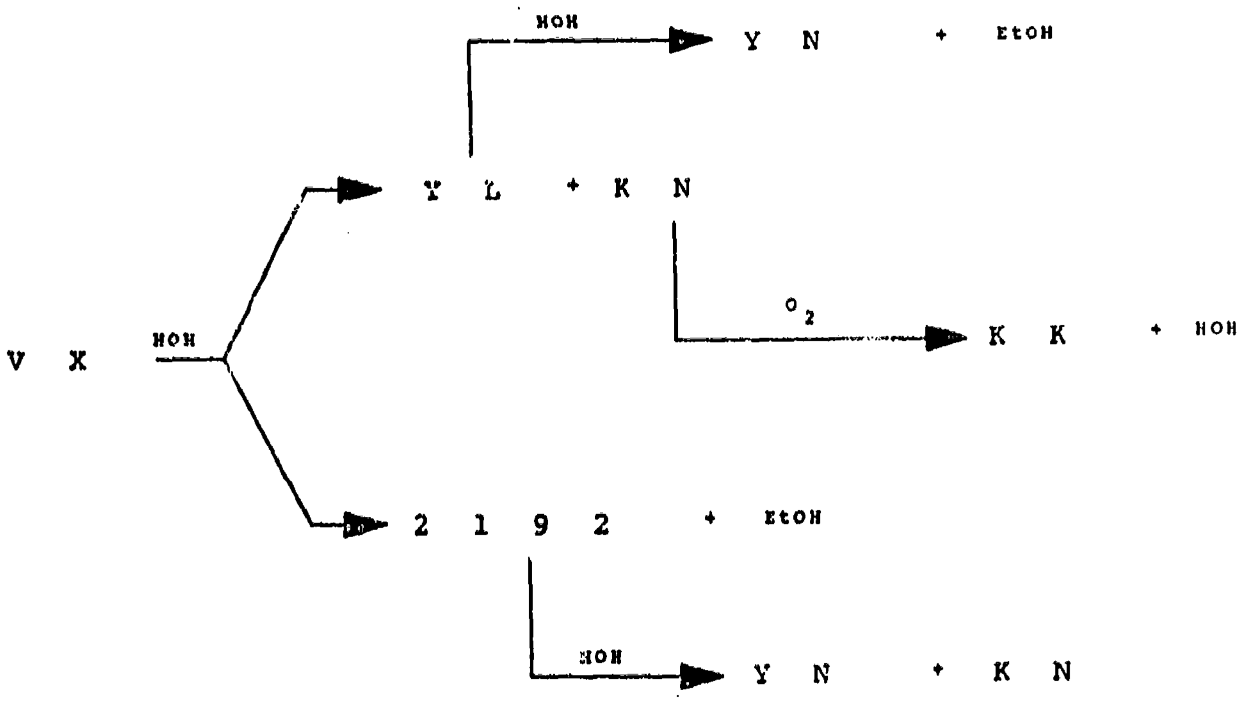
The mode of VX decay in the alkaline test area soil is through alkaline hydrolysis. The rapidity of this hydrolysis was demonstrated in an experiment in which test area soil samples were contaminated with agent at the one percent level. One set of soil samples was oven dried prior to contamination and the second set contained one percent water. The samples were stored in closed flasks at 25 ^{o}C. It is apparent from the results shown in Figure 11 that even a small amount of water is adequate to promote rapid hydrolysis of VX in this soil.

The major decomposition products that are possible through the alkaline hydrolysis of VX are shown in Figure 12. The decomposition products were detected and identified in the soil samples using thin layer chromatography. However, not all of these decomposition products are found in every sample of aged contaminated test area soil. Some products appear only as the soil sample ages longer.



□ 0.0 percent soil moisture
◇ 1.0 percent soil moisture

Figure 11. Recovery From Test Area Soil Containing 1% VX. (Closed Flask Experiments at 25 °C).



VX = O-ethyl s-(2-diisopropylaminoethyl
methylphosphonothiolate
YL = o-ethyl methylphosphonic acid
KN = 2-diisopropylaminoethylthiol
YN = methylphosphonic acid
KK = bis (2-diisopropylaminoethyl) disulfide
2192 = S-(2-diisopropylaminoethyl)
methylphosphonic acid

Figure 12. Alkaline Hydrolysis of VX

The results of mouse toxicity studies with products from alkaline hydrolysis are listed in Table 8. These results indicate that these decomposition products are from 6,579 to 13,158 times less toxic than is VX.

Table 8. Intraperitoneal Toxicity (LD_{50} in Mice) of VX and Some Decomposition Products From Alkaline Hydrolysis

O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX)	0.038 mg/kg
Methylphosphonic acid (YN)	500 mg/kg
O-ethyl methylphosphonic acid (YL)	250 mg/kg
2-diisopropylaminothylthiol (KN)	375 mg/kg

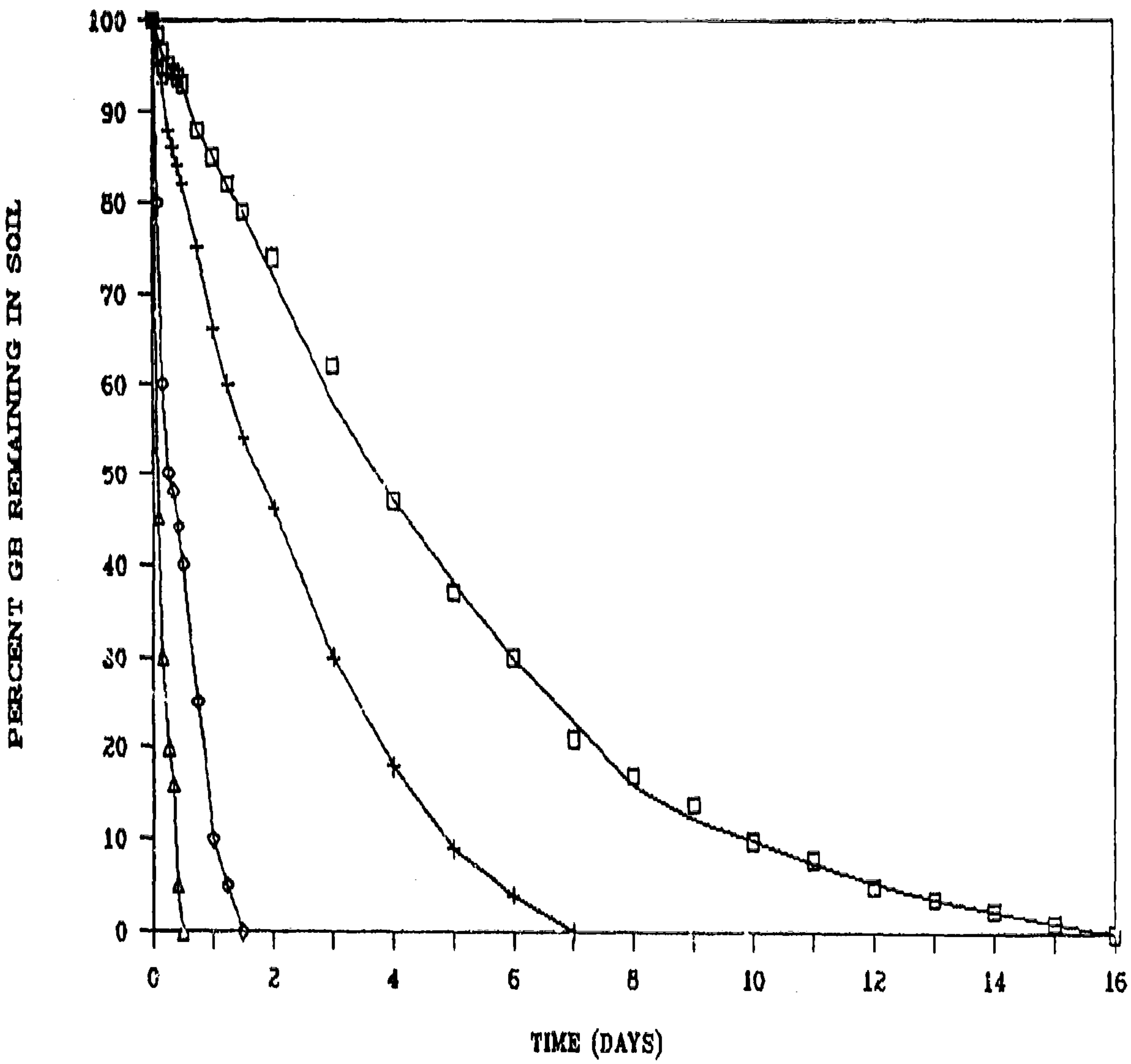
2.2.1.2 <u>The Decay of GB in Soils</u>

A. The Decay of GB in Test Area Soil

(1) <u>Closed-flask experiments</u>

The rate of loss of GB from the shadscale-gray molly-greasewood soil prevalent in the test areas is summarized in Figure 13 for 1,000 $\mu g/g$. Even with the winter conditions of 10 ^{o}C soil temperature and 5 to 10 percent moisture in the soil, the half-life of GB is only 2 days. A more complete description of the fate and persistence of GB in soil (closed flask) has been presented elsewhere (reference 2). The difference in the decay rate of GB in a variety of DPG soils is shown in Figure 14.

The rapid loss of GB in pickleweed soil is probably due to the extremely high soluble salt content (21 percent) and the pH of the soil. The clay content of the soil is quite high but the clay mineralogy was not determined. Also, the cation exchange capacity was not measured. However, it was observed that water readily penetrated the pickleweed soil as shown by the fluctuation of the water table in the soil area and the deposition of salts on the surface of the soil due to capillary action. This indicates the clays do not contain multiple lattices and are not "swellable".



□ 1 percent scil moisture, 10°C
+ 21 percent soil moisture, 10°C
◊ 1 percent soil moisture, 40°C
Δ 21 percent soil moisture, 40°C

Figure 13. Observed Decay of GB on Test Area Soil in the Absence of Wind at an Applied Concentration of 1CJ0 µg GB/g Soil.

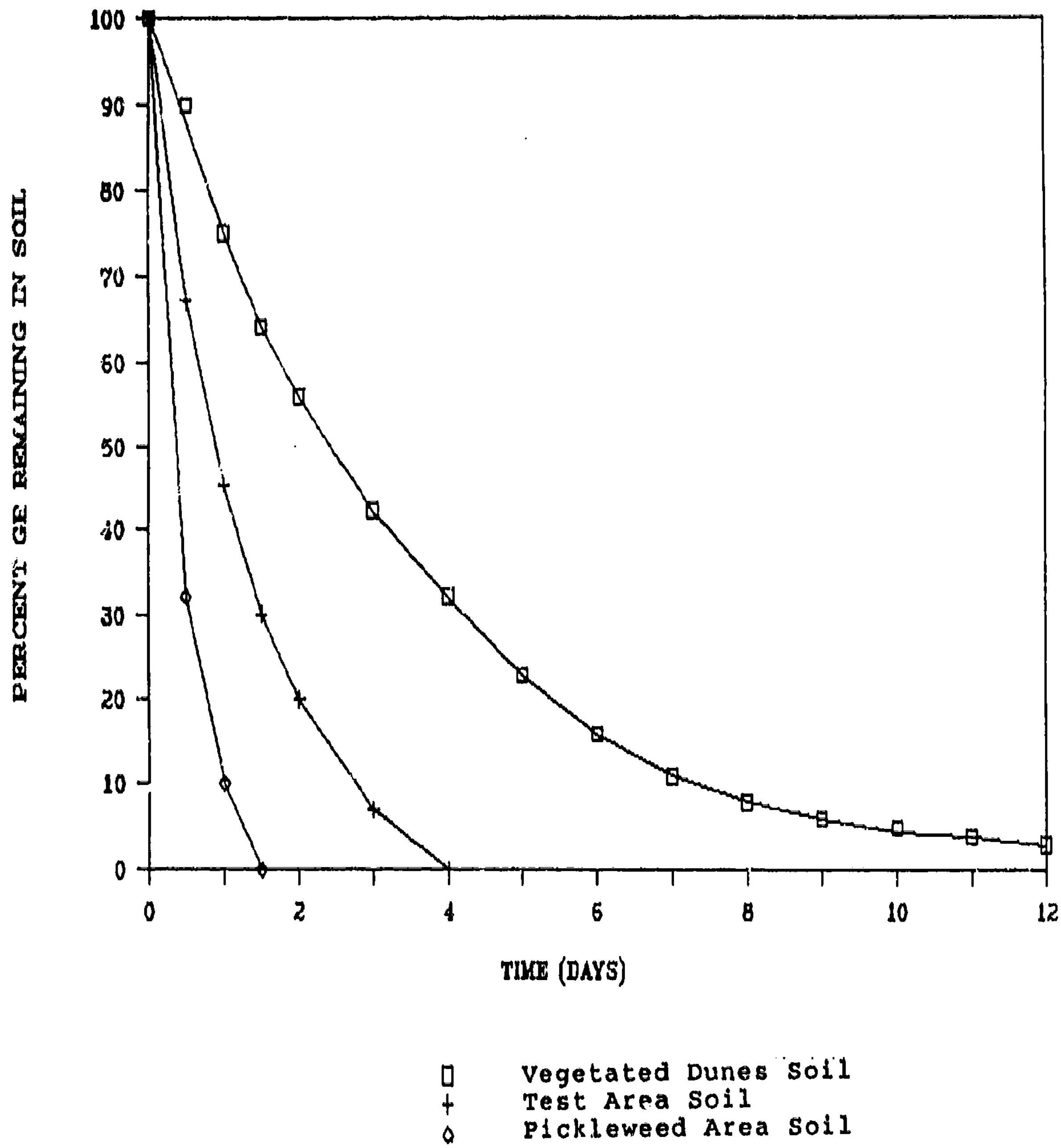


Figure 14. Difference in GB Decay in Various DPG Soils. (5% Moisture, 10 °C, Vegetated Dunes = 316 μg GB/g, Test Area Soil = 398 μg GB/g, Pickleweed Area = 460 μg GB/g).

The clay mineralogy and the cation exchange capacity (30 meg/100g) of the Shadscale-Gray Molly-Greasewood was determined as was the soluble salt content (1.74 percent). The rate of loss of GB from this soil was about one-half that found with pickleweed soil. But, the slower rate is probably due to a much lower soluble salt content rather than the moderately high cation exchange capacity. This view is further substantiated by the results reported elsewhere (reference 2). When the soil was protected from water vapor, the agent recovery was high (90 percent) and remained so for 5-6 days. However, the addition of a small amount of water caused an immediate and rapid decay of GB. This information indicates that the agent is lost primarily through alkaline hydrolysis. The vegetated-dune soil studies revealed that GB will persist at least twice as long as in the shadscale-gray molly-greasewood soil. This soil is nearly all sand with very low soluble salt and clay contents.

(2) Open-container experiments

The analysis of variance showed that the half-life of GB on test area soil was significantly affected by temperature at the 99 percent confidence level, and by wind velocity and the velocity x temperature interaction at the 95 percent level. The quantitative relation between these variables in the experiment is given by equation 3.

$$t_{1/2} = 11.1525 - 1.6542V - 0.2602T + 0.0398VT \qquad (3)$$

where $t_{1/2}$ is the half-life (days), V is the wind velocity (miles per hour), and T is the temperature (^{o}C).

This equation was used to prepare Table 9. These results are presented in Figure 15.

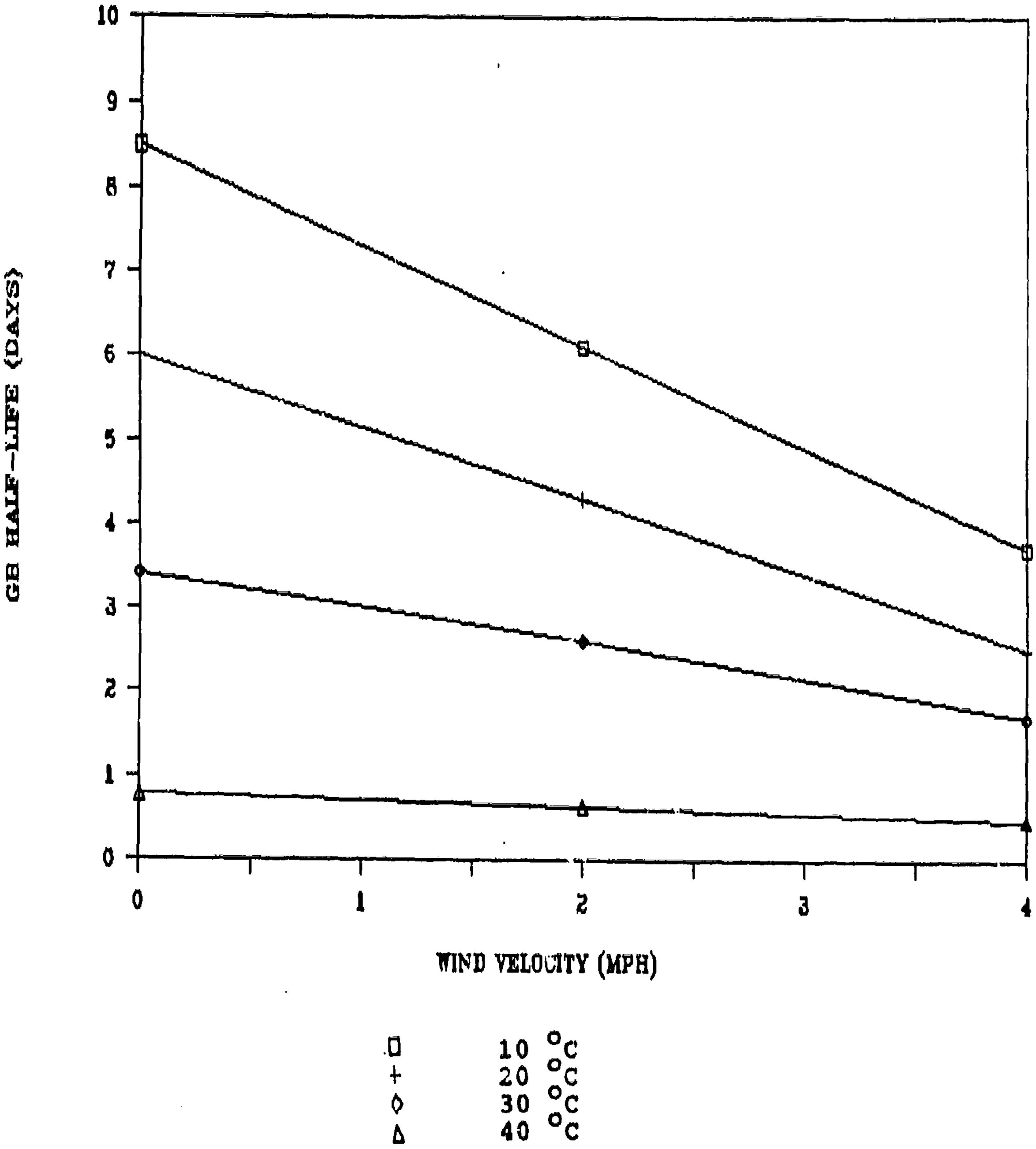


Figure 15. Half-Life of GB in Test Area Soil in Open Air.

Table 9. Calculated Half-Life (days) of GB on Test Area Soil in Open Air

Temp. (°C)	Initial Conc. ($\mu g/g$)	Wind Velocity (mph) 0, Moisture Content of Soil (Percent) 1.2	0, 4.3	4, 1.2	4, 4.3
10	100	8.6	8.6	3.5	3.5
	1000	8.6	8.6	3.5	3.5
20	100	5.9	5.9	2.5	2.5
	1000	5.9	5.9	2.5	2.5
30	100	3.3	3.3	1.5	1.5
	1000	3.3	3.3	1.5	1.5
40	100	0.7	0.7	0.5	0.5
	1000	0.7	0.7	0.5	0.5

B. The Decay of GB in Soils from Other States

Three different soils collected near Fort Greeley, Alaska, and composite samples of soils from Rocky Mountain Arsenal, Colorado, and from Edgewood Arsenal, Maryland, were briefly studied. Sample 1 from Ft. Greeley was from the top three inches of soil in an aspen forest. This soil was a sandy loam high in organic matter. Sample 2 was from the same location, but was a sand taken at a depth of 12-16 inches. Sample 3 was a composite sandy loam taken in a spruce forest.

The Rocky Mountain Arsenal soil was a composite of small samples taken from the surface around the arsenal. The Edgewood soil was a composite of samples from Carroll Island.

The soils were moisture-conditioned and contaminated with GB as described for the closed-flask soil experiments (2.1.1.2, A). The results obtained by periodically extracting and analyzing samples yielded the half-lives listed in Table 10. The rate of decay of GB in these soils is similar to that found in Dugway test area soil and vegetated dune soil.

Table 10. Decay of GB in Selected Soils (Closed Flask)

Soil Source		Application Level (μg GB/g)	Soil Moisture (Percent)	Temperature (°C)	GB Half-Life (days)
Ft. Greeley	1	250	5	25	0.2
	2	250	5	25	0.9
	3	250	5	25	1.1
Rocky Mtn Arsenal		7	10	10	1.5
		7	10	40	0.5
		700	10	10	1.4
		700	10	40	0.4
Edgewood Arsenal		7	10	10	3.0
		7	10	40	0.2
		700	10	10	1.2
		700	10	40	0.6

C. The Mode of Decay of GB in Soil

The mode of GB decay in DPG soils is through alkaline hydrolysis if the sample is protected from the wind (closed flask). If wind is present, the major route by which GB is lost (decay) is through evaporation. This has been extensively studied and reported elsewhere (reference 2). The results are summarized in Figure 16.

2.2.1.3 <u>The Decay of Malathion in Soil Compared to VX and GB</u>

Tons of malathion are sprayed onto our plants and farm soils annually. We eat food that has been sprayed with malathion, breathe air while it is being sprayed for mosquito control, and contact the soil contaminated with malathion. Since malathion is treated as a low persistency anticholinesterase insecticide, experimentally comparing the residual toxicity of

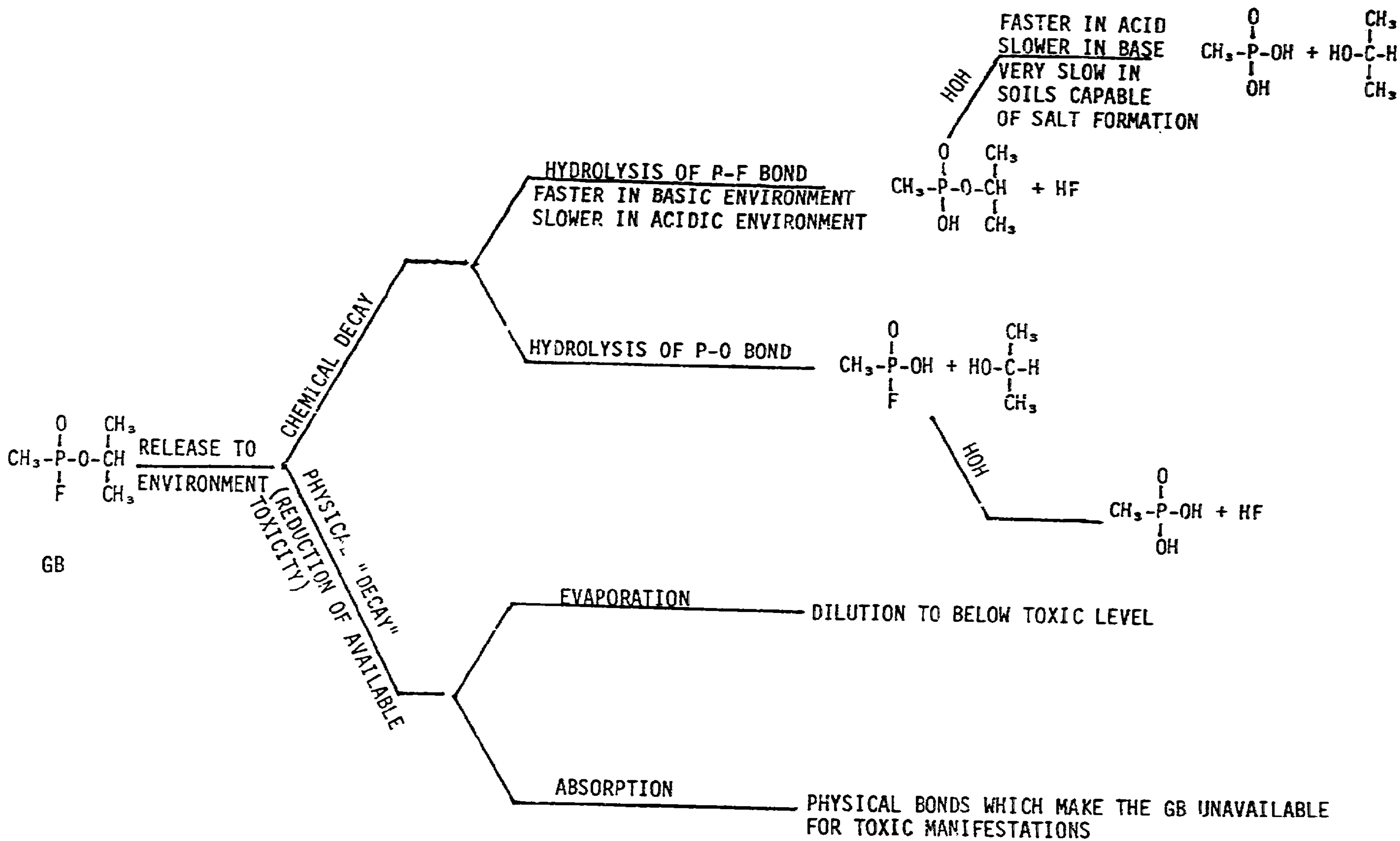


FIGURE 16. PRINCIPAL MODES OF GB LOSS

malathion with those of GB and VX should help to make a more realistic assessment of the potential hazard from introducing these materials into the local environment.

The half-lives for these three nerve chemicals in test area soil over a range of temperatures and soil moisture contents are shown in Table 11 using open containers.

Under the summer conditions simulated in the open container experiments (40 ^oC, 1.2 percent soil water, 0 mph wind), if a soil plot was contaminated with 192,500 mg of malathion, or 192.g, which is twice the oral LD_{50} for humans, and other plots of soil were contaminated with this same quantity of GB and VX, after 20 days 93,000 mg (which is one oral LD_{50} for man) of malathion or, one-half of the original amount, will remain; however, only 0.184 mg of GB or VX will remain which is 1.1×10^{-4} of the oral LD_{50} for man for GB and 1.9×10^{-2} of the percutaneous LD_{50} for VX. Although the nerve agents GB and VX are approximately 10,000 times more toxic than malathion, after a relatively short time on soil, the residual toxic hazard from the commonly used insecticide malathion can be many times greater than that from GB or VX.

Table 11. Comparison Between Malathion, GB, and VX on Soil in Open Containers(a)

Wind Speed (mph)	Temp (^oC)	Half-Life on Test Area Soil (Days): 1.2% H_2O Mal	1.2% H_2O GB	1.2% H_2O VX	4.3% H_2O Mal	4.3% H_2O GB	4.3% H_2O VX
0							
	10	454	9.1	16.5	60	4.2	15
	40	20	1.0	1.0	6.6	0.46	2.0
4							
	10	281	5.8	14.0	44	1.2	13
	40	18	0.85	0.50	5.6	0.14	1.5

a. Averaged over 100 and 1,000 µg/g soil. Malathion and VX both usually gave the same half-life at 1,000 µg/g as they did at 100 µg/g.

Under conditions that approximate spring or fall (10 °C, 4.3 percent soil water), the malathion will have gone through one half-life in 60 days. During this time, if starting with weights of malathion, GB, and VX that are twice the oral LD_{50} for malathion, the GB will have gone through 14.3 half-lives, (or 9.6 mg will remain), which is 5.7×10^{-3} of the oral LD_{50} for man for GB. During this same time period, the VX will have gone through only 4 half-lives, or 12,030 mg of VX remains in the soil. When such a large quantity of VX is initially placed on the soil (192.5 g), the residual agent that remains probably could partition itself between the soil and the skin to cause death.

The results for the 2x2x2x2 factorial experiment with malathion in test area soil in open air are presented in Table 12. Equation 4 was derived from this data and used to prepare Figures 17 and 18.

Table 12. Calculated Half-Life (days) of Malathion on Test Area Soil in Open Air

Temp (°C)	Initial Conc (µg/g)	Wind Velocity (mph) 0, Moisture Content of Soil (Percent) 1.2	0, 4.3	4, 1.2	4, 4.3
10	100	454.	60.	281.	44.
	1000	454.	60.	281.	44.
20	100	309.	42.	193.	31.
	1000	309.	42.	193.	31.
30	100	165.	24.	106.	18.
	1000	165.	24.	106.	18.
40	100	20.	7.	18.	6.
	1000	20.	7.	18.	6.

$$t_{1/2} = 800.10484 - 77.66054\ V - 167.96237\ W + 16.80045\ VW - 19.36081\ T + 1.92584\ VT + 4.08817\ WT - 0.41785\ VWT \quad (4)$$

where $t_{1/2}$ = half-life (days), V = wind velocity (mph), W = water content of soil (percent), and T = temperature (C).

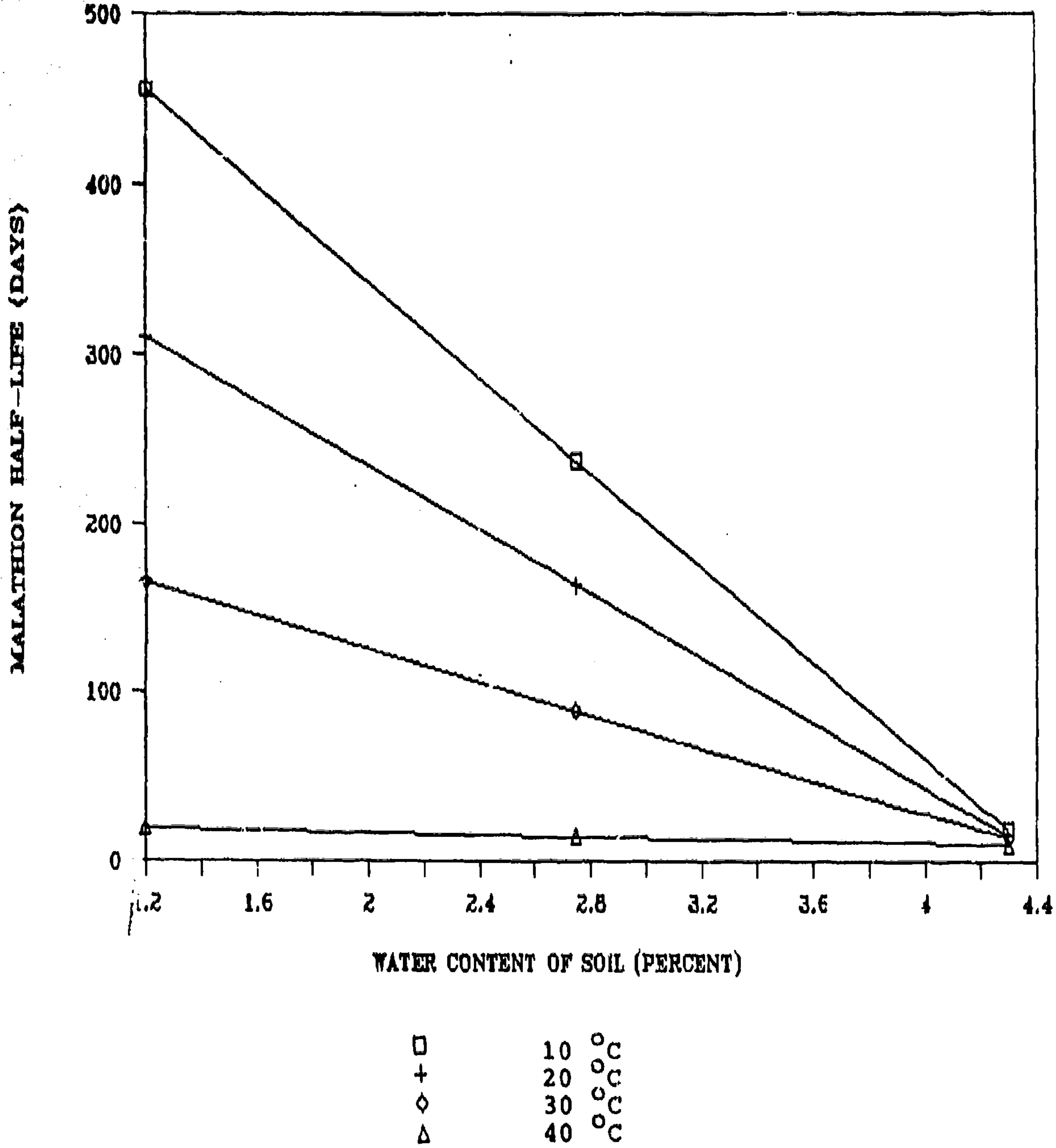


Figure 17. Persistence of 100 to 1000 μg/g Malathion in Test Area Soil Exposed to 1.2 mph Wind.

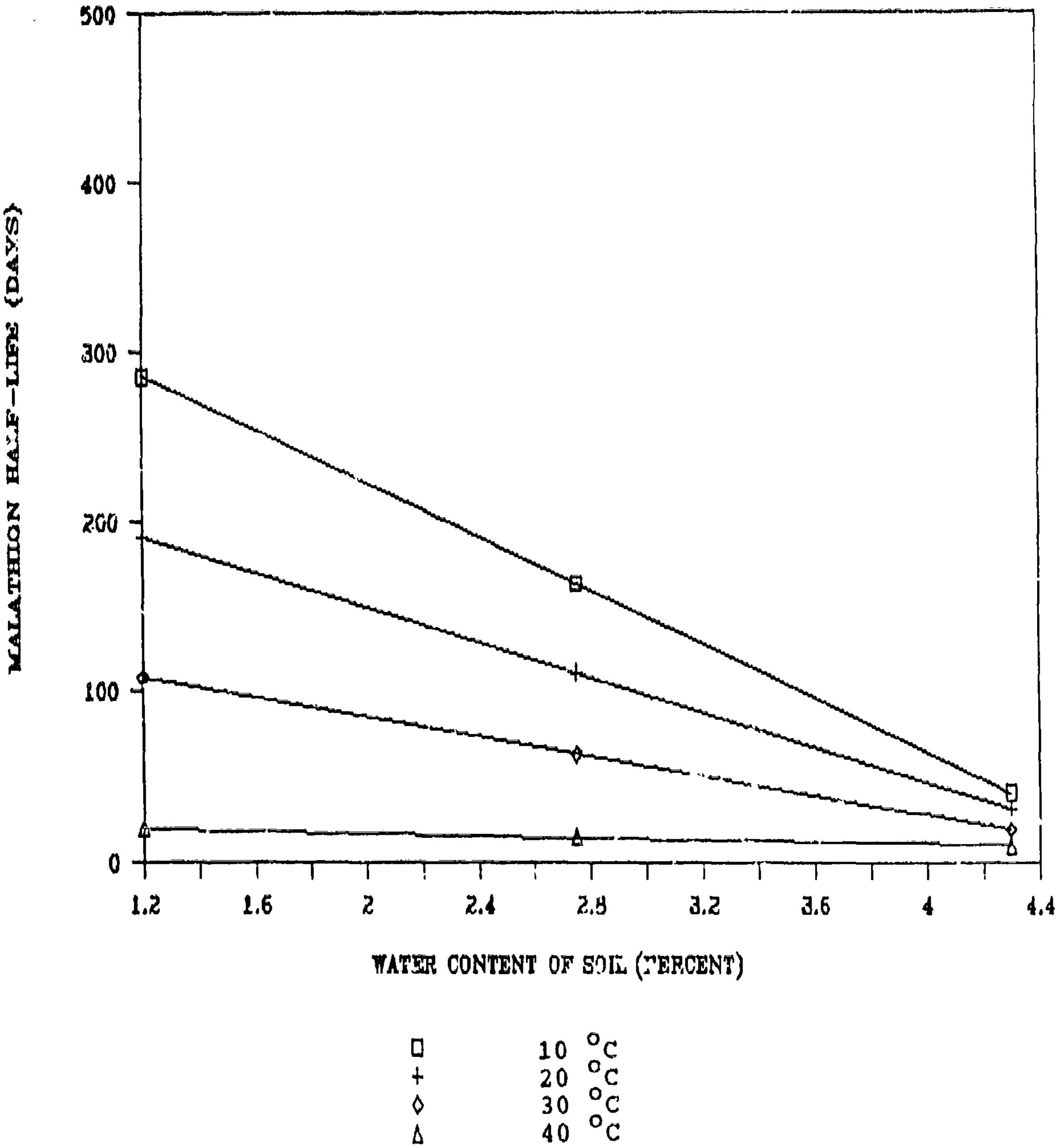


Figure 18. Persistence of 100 to 1000 μg/g Malathion in Test Area Soil Exposed to 4.0 mph Wind.

Wind velocity, water content, and temperature all were significant at 99 percent level.

2.2.2 SURFACE WATER INVESTIGATIONS

2.2.2.1 The Decay of VX in Surface Waters

Both the White Rock pond and the Brackish pond were analyzed for selected cations, conductivity, and pH. The results are given in Table 13.

The analysis of variance of the results from the factorial experiment showed that varying the contamination level from 100 to 1000 μg VX/mL water did not have a statistically significant effect on the half-life of VX in Dugway surface ponds. The effect of temperature was significant at the 99 percent level. The significant effect of temperature is evident in Figure 19 (The half-life at 10 ^{o}C is 29 days; the half-life at 40 ^{o}C is 0.7 days). Equation 5 was derived and used to prepare decay plots, Figure 20, and half-life predictions, Table 14.

$$t_{1/2} = 38.433 - 0.943T \text{ where } t_{1/2} = \text{half-life (days) and } T = \text{temperature } (^{o}C). \quad (5)$$

Table 13. Analysis of Pond Water

Source	pH	Conductivity (mho)	Cation Concentration (μg/mL) Mg	Ca	K	Na
White Rock Pond	8.8	3.7x10	9.5$\pm$0.5	25.0$\pm$1.5	16.0$\pm$1.0	15.0$\pm$1.0
Brackish Pond	8.8	6.2x10	4.5$\pm$0.5	9.5$\pm$0.5	8.0$\pm$1.0	40.0$\pm$5.0

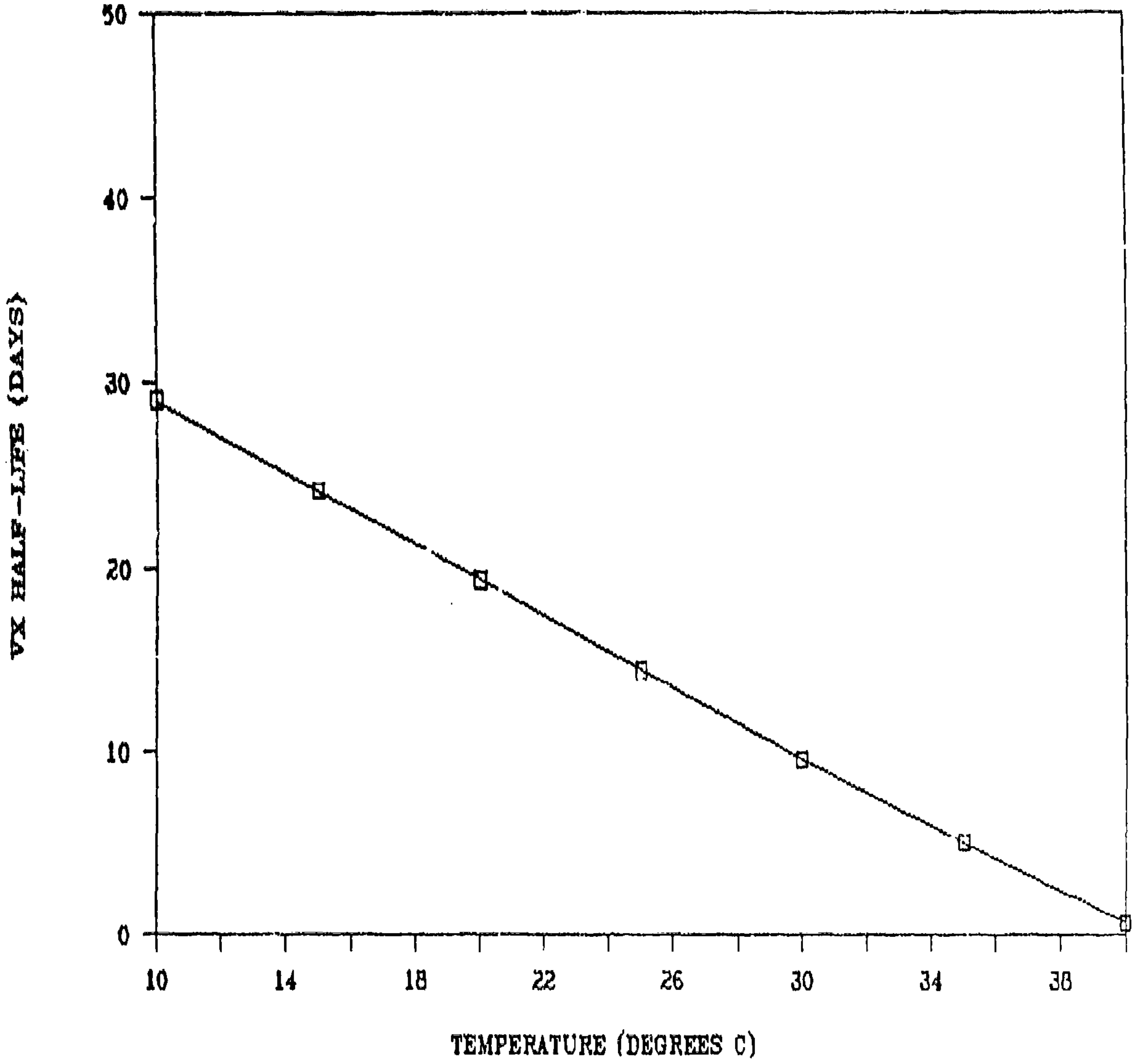


Figure 19. Half-Life of VX in Test Area Pond Water at 100 and 1000 µg/mL.

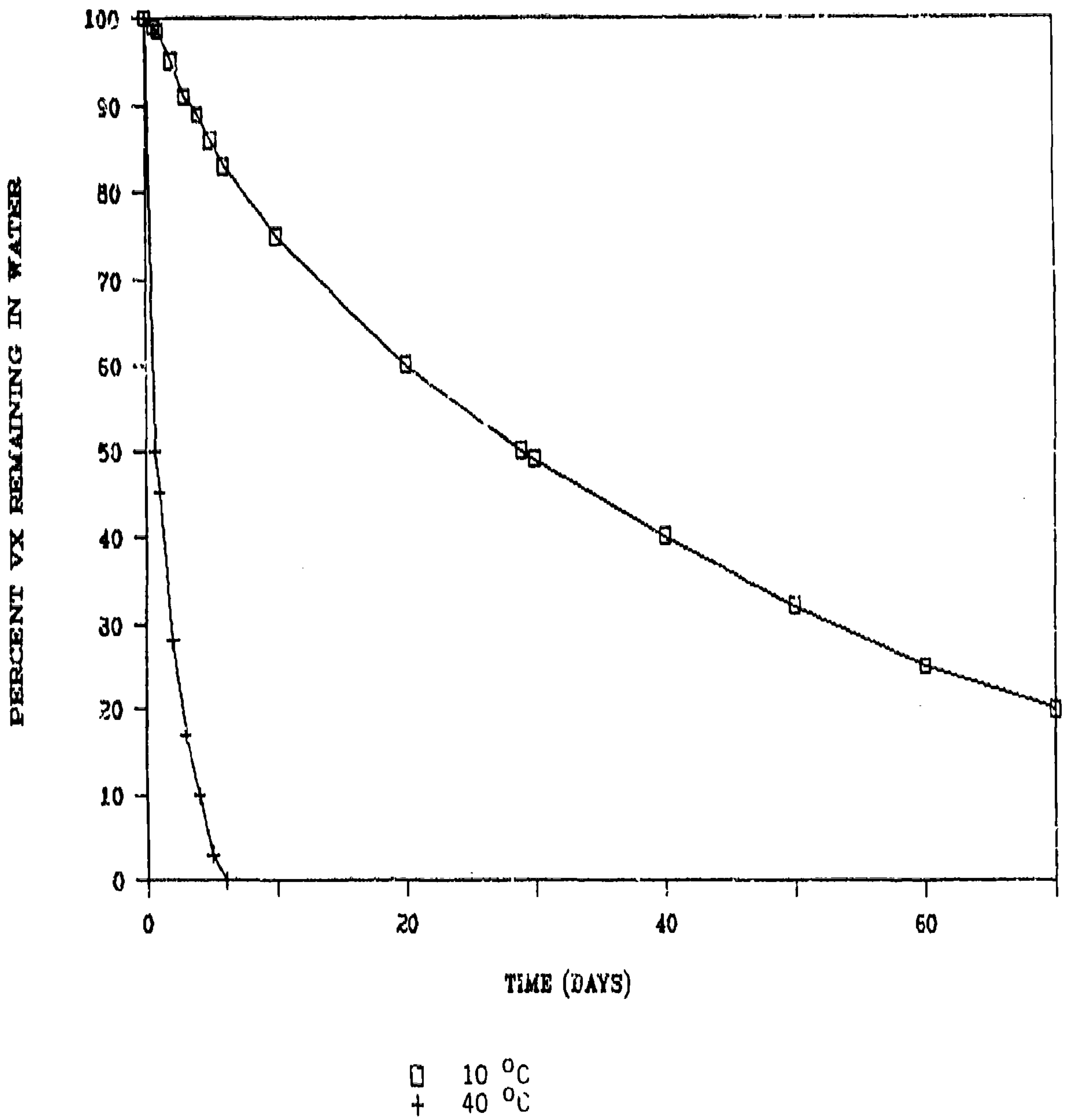


Figure 20. Persistence of VX in Pond Water (100 and 1000 µg/mL).

Table 14. Calculated Persistence of 100 to 1,000 $\mu g/mL$ VX in Test Area Pond Water

Water Temperature (°C)	VX Half-Life (days)
10	29.0
40	0.70

It was concluded that VX would disappear quite rapidly from a casual pond in the test area. Although the persistence is moderate at 10 °C, even in the springtime the water could reach 20 to 30 °C because of solar warming of the soil and the shallowness of the ponds.

The analysis of propylated samples revealed that YL, KK, and a small amount of YN was present in the samples that had partially decayed.

2.2.2.2 The Decay of GB in Surface Waters

The rate of decay of GB in both White Rock pond and Brackish pond waters was temperature and concentration dependent. The higher GB concentration had the longer half-life and the higher temperature gave a shorter half-life. The results are summarized from an earlier study (reference 2) and shown in Table 15.

Table 15. Observed GB Half-Life in Pond Water

Temp. (°C)	GB Half-Life (days)			
	White Rock Pond		Brackish Pond	
	10 $\mu g/mL$	100 $\mu g/mL$	10 $\mu g/mL$	80 $\mu g/mL$
10	0.92	3.50	1.26	4.71
20	0.08	1.04	0.08	0.98
30	0.03	0.41	0.02	0.33
40	0.07	0.29	0.04	0.25

2.2.3 VEGETATION INVESTIGATIONS

2.2.3.1 The Decay of VX in Plants

A. The Decay of VX in Dead Plant Materials

An analysis of variance showed that changing the initial VX concentration from 100 to 1,000 µg/g had no significant effect on the persistence, while the moisture content of the grass and the temperature were significant at the 99% level. The magnitudes of their effects are evident in Table 16. The effect of temperature and water interaction is seen to be less at 40 °C than at 10 °C; this (Water) x (temperature) interaction is significant at the 95% level according to ANOVA. The ANOVA also showed that the effect of temperature is non-linear, with a square term significant at the 99% level. This curvature is seen in the plots of Figures 21 and 22.

The magnitudes of the factor effects and the interactions yielded the following regression equation, which allows calculating the half-life of VX on dead cheat grass for any combination of temperature, moisture content, and VX concentration under the range of conditions used for this experiment:

$$t_{1/2} = 56.29578 - 1.67800W - 2.63009T + 0.03953WT + 0.03251T, \qquad (6)$$

where $t_{1/2}$ = half-life (days), W = water content (percent), and T = temperature (°C).

The loss of VX from dead grass in the presence of ventilation was not studied because some of the milled dried grass blew out of the small petri dishes used in the wind chamber.

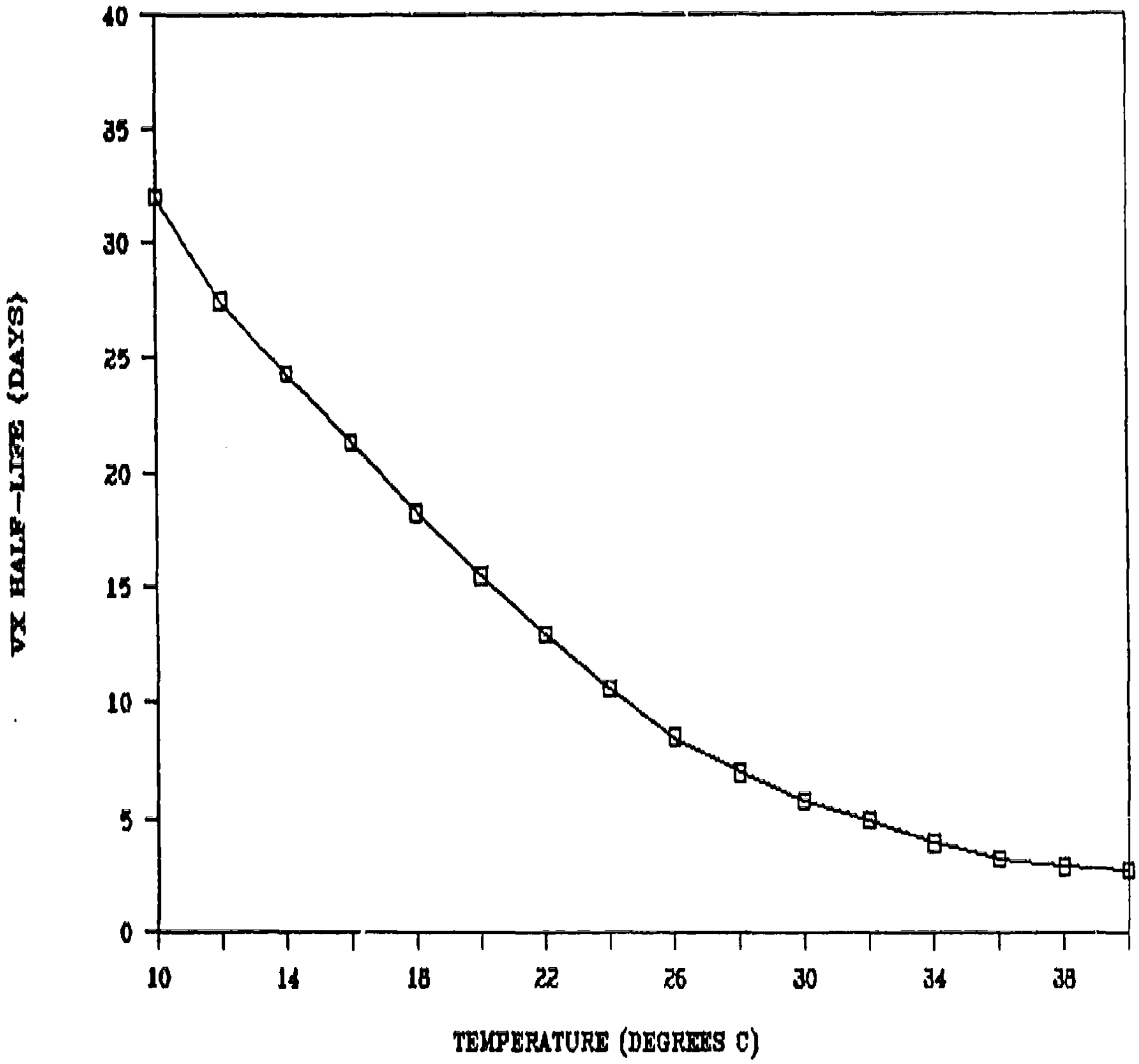


Figure 21. Persistence of 100 to 1000 μg/g VX on Dead Cheat Grass Containing 1% Water.

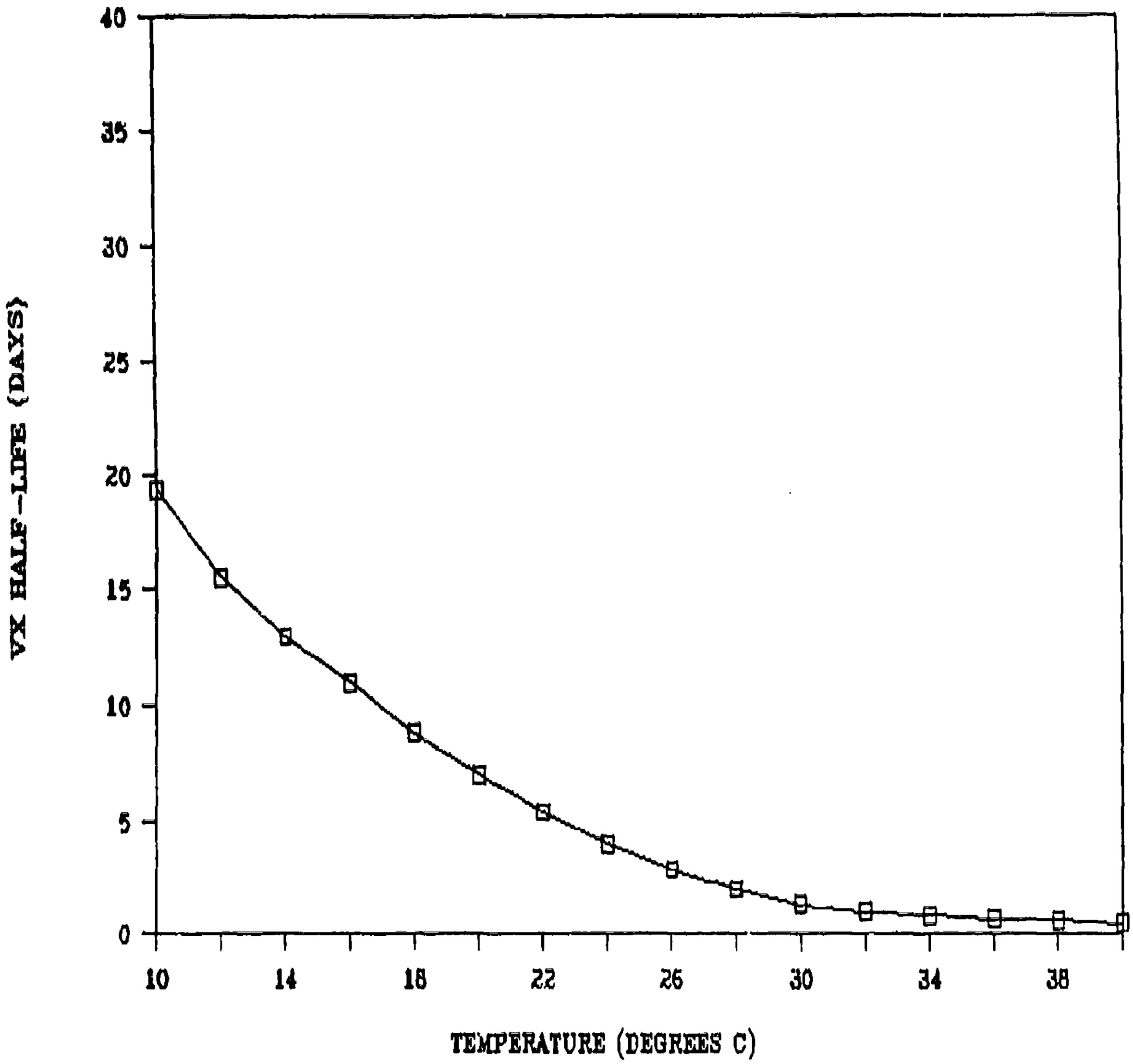


Figure 22. Persistence of 100 to 1000 μg/g VX on Dead Cheat Grass Containing 11% Water.

Table 16. Half-Life (days), of VX on Dried Cheat Grass, Closed Flask

Temp. (^{o}C)	Initial Conc. (μg/g)	Moisture Content of Grass (percent) 1.0	3.0	5.0	7.0	9.0	11.0
10	100	32.0	29.4	26.8	24.3	21.7	19.1
	1,000	32.0	29.4	26.8	24.3	21.7	19.1
15	100	23.1	20.9	18.7	16.6	14.4	12.2
	1,000	23.1	20.9	18.7	16.6	14.4	12.2
20	100	15.8	14.0	12.3	10.5	8.7	6.9
	1,000	15.8	14.0	12.3	10.5	8.7	6.9
25	100	10.2	8.8	7.4	6.0	4.7	3.3
	1,000	10.2	8.8	7.4	6.0	4.7	3.3
30	100	6.2	5.2	4.2	3.2	2.2	1.2
	1,000	6.2	5.2	4.2	3.2	2.2	1.2
35	100	3.8	3.2	2.6	2.0	1.4	0.8
	1,000	3.8	3.2	2.6	2.0	1.4	0.8
40	100	3.0	2.8	2.6	2.4	2.2	2.0
	1,000	3.0	2.8	2.6	2.4	2.2	2.0

VX was also applied to samples of dead greasewood, juniper bark, and budsage in very limited experiments. The samples were contained in open Erlenmyer flasks and stored at room temperature. These experiments were conducted for comparative purposes with the cheat grass experiments. The concentration of the applied agent had little effect on the rate of decomposition. Thus, the samples in each agent application level were averaged together. The results are shown in Table 17. The results were similar to the open flask cheat grass results.

Table 17. The Observed Half-Life of VX in Dead Vegetation (Open Containers)

Sample Identification	Agent Application Levels ($\mu g/g$)	Environmental Factors	Half-Life (days)
1. Greasewood	100 and 1,000	Dead vegetation, 6.8 percent moisture, 25 ^oC.	11-16
2. Budsage	100 and 1,000	Dead vegetation, 4.4 percent moisture, 25 ^oC.	14-22
3. Juniper Bark	100 and 1,000	Dead vegetation, 6.4 percent moisture, 25 ^oC.	14-21

B. The Decay of VX in Freshly Harvested Plants

VX was applied to freshly harvested samples of budding greasewood samples and dormant winterfat and sagebrush samples. The samples were stored at room temperature and contained in open flasks. The results show that VX in living plant samples decays more rapidly than in dead plant samples. It was concluded that the increase in the rate of VX decomposition (disappearance) was due either to the increase in moisture content or ongoing metabolic processes that can either utilize VX or catalyze decomposition hydrolysis. The experiments are only fragmentary and intended to highlight differences in VX persistence in living plant specimens compared to dead specimens. They are not intended to be a definitive study. The results are shown in Table 18.

Table 18. The Observed Half-Life of VX in Freshly Harvested Plants, (Open Containers)

Sample Identification	Agent Application Levels ($\mu g/g$)	Environmental Factors	Half-Life (days)
1. Greasewood	100-1000	Budding vegetation, 31 percent moisture	4.5
2. Winterfat	70	Dormant winter specimen, moisture undetermined	5
3. Sagebrush	70	Dormant winter specimen, moisture undetermined	5

C. The Decay of VX in Actively Growing Plants

(1) Topical application studies

A series of investigations were initiated in which the fate of agent topically applied to plants was studied. Twenty-four beans were planted in a closed vermiculite bed and watered with a liquid nutrient. After development of primary leaves, one microliter of VX was applied to the plants separately at each of the following locations: (a) Apical meristem, (b) stem just above the cotyledon, and (c) at the apex of one of the primary leaves. At the time of application, one microliter of agent was also placed in 10 milliliters of methanol to determine the initial purity of the agent. The purity was found to be 94 percent.

A set of plants were harvested 48 hours after application. These plants were subdivided into various sections, e.g., leaves, stems, etc. Other sets of plants was harvested during mid-vegetative growth, or approximately 5, 9 and 13 days after contamination.

Other sets of plant samples was taken 17 and 22 days after application of VX. The plants appeared to be nearing flowering.

All sample extracts were submitted for gas chromatographic and enzymatic analysis. Results of gas chromatographic and enzymatic analysis indicate good agreement between the two analytical methods.

The topically applied VX appeared to be absorbed quite rapidly by the plants, especially by the leaves. Plant damage was observed. This is discussed in detail below.

When the agent was applied to the apical meristem, vegetative growth (cell division) and formation of new plant tissue was disrupted. Apical dominance was broken and two new buds were formed on either side of the original stem. Only after formation of the new meristems did vegetative growth resume at the point of application. Very little agent was translocated from the originally contaminated apical meristem to other portions of the plant during 22 days of growth. All of the original weight of agent appeared to be lost. The decomposition product YL was found but it constituted only a small percentage of the original mass of VX applied to the plant. Whether VX is lost or converted to decomposition products which may be utilized by the plant could not be determined by this experiment. The elimination of the VX by the total plant is shown in Figure 23.

When agent was applied to one of the two primary leaves, the leaf discolored, became flaccid and eventually abscised. Most of the agent remained in the contaminated leaf. Only a very small quantity was translocated to the other primary leaf and none was found in other plant parts. After 9 days, only 20 percent of the agent remained and after 22 days essentially all of the agent had disappeared. The YL content in the leaf increased with time but accounted for only a small fraction of the amount of VX originally applied. The results are also shown in Figure 23.

The stem was contaminated just above the cotyledons of the plant. The stem showed discoloration and gross shrinkage at the point of application 24 hours later. The vascular system was not disrupted, however, and the flow of nutrient from the root to the aerial portions of the plant continued. No difference in overall plant vigor was observed between the stems of the contaminated plant and the control plants. The agent moved rapidly from the stem and accumulated mainly in the primary leaves. Only a small amount of agent was found in new growth or upper stem. Nothing was found in the hypocotyl or roots. After 22 days, less than one percent of the original weight of agent was present in the plant. The results are shown in Figure 23.

It appears as if the YL arises primarily from in situ hydrolytic decomposition of agent. However, there are

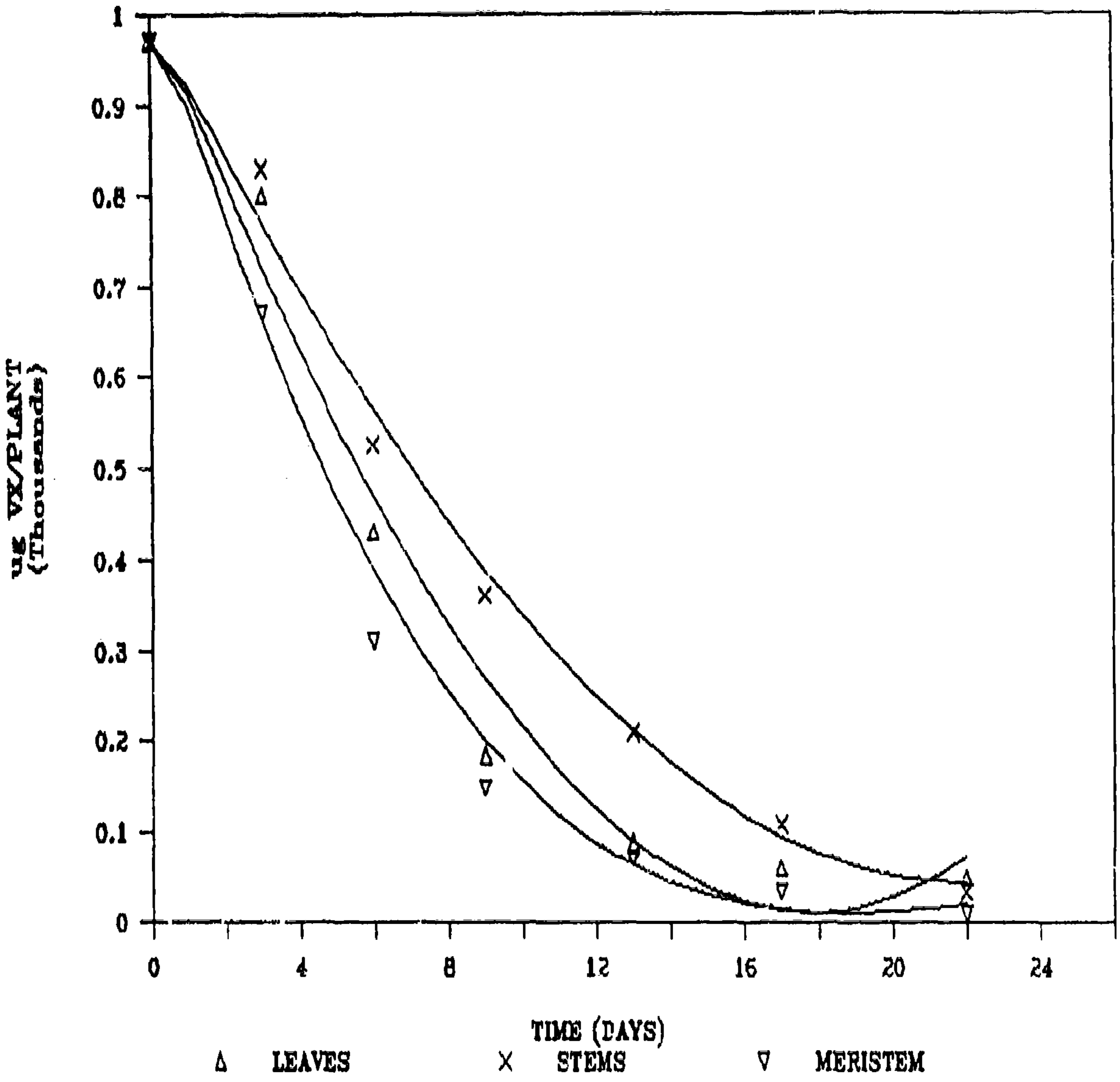


Figure 23. The Elimination of VX From Bean Plants Topically Contaminated at Different Locations on the Plant.

preliminary indications that the YL can be transported in certain parts of the plant and during certain phases of growth. For example, during the time that the apical meristem contaminated plants were forming new meristems, considerable agent and YL was found in the original meristem. The agent content however, was very low in the new meristem while the YL was higher than the agent. The rate of conversion of VX to YL normally found in the various plant sections was much different in this phase of plant growth, indicating possible translocation of YL.

(2) Uptake Studies

Several brief experiments were run which tested the affect of VX upon the various stages of germination and are described below.

A brief description of the germination processes is in order at this point. The resumption of active growth during germination of the seed is initiated by a marked swelling of the seed (embryo) caused by imbibition of water from the surrounding nutrient/soil by various tissues within the seed. This usually results in the rupture of the seed coat and the emergence of the hypocotyl which contains the primary root. The imbibition of water increases permeability of the seed to oxygen and carbon dioxide. Enzyme activity increases and food stored in the endosperm or cotyledon is digested or solubilized and the soluble products translocated toward the growing points of the embryo. The bean does not contain an endosperm but the food is stored in two large thick cotyledons. After the seed swells as a result of water imbibition, the first sign of bean germination is the emergence of the rudimentary root (radicle). The young root grows downward in the nutrient/soil producing lateral roots and root hairs. The hypocotyl or stem structure between the radicle and cotyledon, elongates pulling the cotyledons upward out of the nutrient into the air where they separated into a horizontal position on either side of the plumule (primary leaves). Photosynthesis is started after the primary leaves are exposed to light and active vegetative growth begins. This gives rise to stem development and formation of foliage leaves.

The uptake of VX during germination by beans and the translocation and fate of this agent within the seed/seedlings were studied to determine if VX inhibited germination prior to the start of photosynthesis (the beans and seedlings were etiolated or grown in the absence of light). The nutrient was contaminated with VX. The application level used was 43 μg VX/mL of nutrient. The solution and a control solution (uncontaminated

nutrient) were transferred into two stainless steel trays each of which contained a wire supporting rack. This rack permitted the bean seed to just touch the surface of the liquid. Weighed bean seeds were placed upon the rack and allowed to germinate. The temperature was maintained at 25 ^{o}C and the contaminated nutrient was maintained at a constant level so as to ensure the liquid would remain in contact with the seeds. The trays were covered with foil to prevent light from reaching the seeds During the germination period, the nutrient was sampled periodically and analyzed for its agent content by enzymatic analysis and by extraction followed by GC analysis. Good agreement was obtained between the two methods for the agent. The VX decomposed quite slowly, half being present 12 days after the start of the experiment. In addition, the GC analysis accounted for the appearance of YL in the nutrient. A good material balance was achieved for the agent when the nutrients were quantitatively analyzed for decomposition products as well as agent.

Some bean seeds were removed at 1 day and 4 days after the initiation of the experiment. They were re-weighed, extracted, and analyzed. The quantity of VX found in the beans at the end of one day was significant (7.7, 4.0, and 7.0 μg for the three replicates). The quantities of VX found in the seeds were comparable to the amount of agent they should have picked up based upon the weight of the nutrient imbibed (0.14, 0.08, and 0.12 g weight increase; the original VX concentration in the nutrient was 43 μg/mL). At 4 days the weight of agent doubled in the seeds (12.0, 15.0, and 8.0 μg). The weight of liquid imbibed increased three-fold. It was concluded that the agent taken up by seeds was due to simple imbibition during the seed germination period and VX did not appear to inhibit germination at the agent concentration used in this experiment.

At 7 days and 11 days additional beans were harvested. Each young plant from each of these series was divided into emergent cotyledons and hypocotyl (including roots). Each plant fragment was extracted and analyzed separately. VX was found in both the cotyledons and in the hypocotyl. The average VX content in the cotyledons showed an increase during the period between 4 and 7 days (13 μg to 21 μg). During this phase of germination, the bean and seedling rely solely on food stored in the cotyledons. This reliance continued until photosynthesis was started. Previous analysis of the seeds indicated considerable imbibition of VX with the water and nutrients. In addition, the primary root developed rapidly (between 4 and 7 days) as did the hypocotyl. The elongation of the hypocotyl pulled the cotyledons out of the nutrient and the primary roots provided a means of nutrient absorption. It was felt this could force utilization of the VX in cotyledons. Instead the VX content in cotyledons did not decrease. This indicates that either the VX

in the cotyledons is not utilized to any significant degree during germination or if so, it is replaced by the VX that is absorbed through the primary root. Also, the agent did not seem to inhibit plant growth (at this agent concentration) because the agent treated seedlings were approximately the same size as the control seedlings.

The agent in the young shoots (combined root and hypocotyl) increased with time of contact with the contaminated nutrient (7 days; 18 μg and 15 μg respectively for two harvested plants: 11 days; 65 μg and 51 μg from two plants). The increase in agent content appears to be due to simple absorption and not an active transport.

At 11 days a number of plants were transferred to uncontaminated nutrient. The plants were exposed to artificial light and aeration of the roots was initiated. They were allowed to grow for 17 additional days. One plant was harvested, and separated into root, stem, and leaf fractions. Each fraction was extracted separately and analyzed. The plant contained agent in all three fractions (roots, stem, and leaves). However, the largest amount was found in the leaves. Thus, after the agent is absorbed by the roots it is translocated to the aerial parts. In addition, the agent is either eliminated from the plant by in situ decomposition or transpiration, or is converted to some other compound by the metabolic processes in the plant. It was also observed that a small amount of the agent associated decomposition products were picked up and translocated by the plants (significant amounts of these materials were present in the nutrient). The small amount of these decomposition products found in the plants may be due to the difficulty the compounds have in passing through the root tip membranes because of their polarity and/or the absence of an active transport mechanism.

The remaining plants were harvested at flowering and after formation of seeds. No agent was found in any plant parts at either of these harvests. The remaining plants were harvested and fed to guinea pigs. This was done to establish if a residual toxic hazard (beside anti-cholinesterases) remained in the plants. Blood samples were taken prior to feeding and at 24 and 72 hours after commencement of feeding (the feeding and blood drawing was done by Life Sciences Division personnel). No depression of the red blood cell (RBC) cholinesterase was found (the Michel delta pH method was used). From this it was concluded that no anti-cholinesterases remained in the plant, and verified the reliability of the chemical extraction and analytical methods.

The affect of VX concentration upon bean plants vegetative growth was studied next. The concentrations of the agent in the nutrient were 250, 500, and 1,000 µg VX/mL of nutrient. Bean seeds were placed on wire racks contained in stainless steel trays previously described. Four trays, three trays containing agent contaminated nutrient and one containing uncontaminated nutrient (control) were used.

VX was rapidly taken up by the bean seeds and seedlings during germination regardless of the agent concentration. But, the agent uptake was not proportional with concentration. It was found that the three concentrations used in these experiments retarded plant growth as compared to the control plants. The 1,000 µg VX/mL nutrient concentration caused more growth retardation than the two lower concentrations. The same was true comparing the 500 µg VX/mL nutrient with the 250 µg VX/mL nutrient and the 250 µg VX/mL with the controls.

When light and root aeration was started at 9 days the amount of VX found in the plants dropped off rapidly regardless of the concentration in the nutrient. After the plants were transferred to uncontaminated nutrient at 18 days the VX concentration in the plants dropped to zero between 29 and 35 days. The results are shown in Figure 24.

Bean plants were sprouted in a nutrient media to study uptake and translocation of YN and YL and to determine if further decomposition of the agent decomposition products occur. (After the YL nutrient solution was prepared analysis indicated the YL had disproportionated resulting in the nutrient containing predominantly WJ and YN.) Plant samples were taken at sprouting, mid-vegetative, flowering, and fruit formation phases of plant growth. Analysis of seeds sprouted in media containing the agent decomposition products indicated imbibition of the decomposition products. After transfer of the seedlings to uncontaminated media, however, there was a decrease in the amount of decomposition products found. In contrast to the uptake and translocation of VX from the roots and its subsequent accumulation in the leaves, the concentrations of YN and WJ were found to remain high in the roots and stems but were much lower in the cotyledons and primary leaves. This indicates that uptake had occurred by the primary roots but the decomposition products either translocated from the roots to the aerial portions at a much slower rate than the agents or they were more rapidly utilized or lost through transpiration. As plant growth continued, transpiration, decomposition and/or use in metabolic cycles caused a decrease in the amount of decomposition products in all segments of the plants (roots, stems, leaves, flowers, and seeds) until they were below the level of detection.

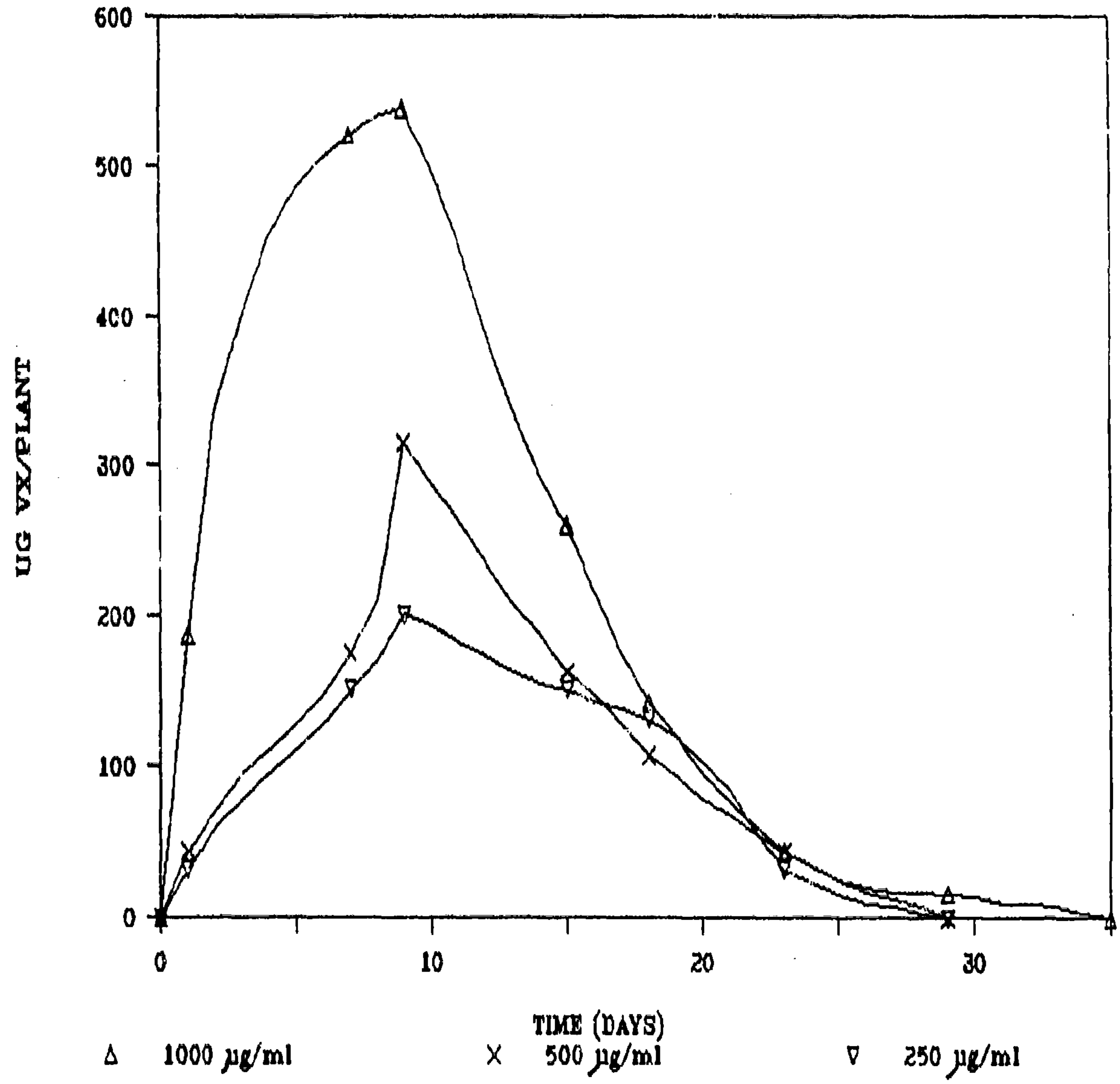


Figure 24. Uptake. Translocation. and Elimination of VX by Bean Plants.

2.2.3.2 The Decay of GB in Plants

A. The Decay of GB in Dead Plant Materials

The results obtained from an extensive study of the persistence of GB in dead cheat grass and budsage (closed flask studies) has been reported elsewhere (reference 2). In summary, the half-life of GB was 1 to 6 days regardless of the moisture content or temperature of these dead vegetation samples. The mode of decomposition and/or loss was through evaporation and/or hydrolysis.

B. The Decay of GB in Actively Growing Plants

Uptake and topical contamination studies with GB have been conducted with bean plants (both unlabeled and labeled GB were used). The uptake, translocation, and elimination of GB by these plants was determined in these studies. The results have been reported in detail (references 2 and 3). The fate of GB in bean plants is summarized here for purposes of completeness of this report.

GB is taken up by bean plants and is rapidly translocated to the aerial portions of the plant accumulating in the leaves. The agent is eliminated by the plant through transpiration and hydrolysis. There were indications that an enzyme may be present in the roots of bean plants that catalyzes the hydrolysis of the agent. When the plants contaminated with GB were placed in uncontaminated nutrient the agent completely disappeared in 8 hours or less. When the plants were topically contaminated on one of the primary leaves the agent stayed at the contamination site and was lost through evaporation, transpiration, and hydrolysis in less than 8 hours. If the plant was topically contaminated on the stem below the primary leaves the GB moved rapidly from the stem and accumulated mostly in the primary leaves. All agent was lost from the plant in 8 hours.

(1) Topical Application Studies

Budsage plants, transplanted from the desert and watered with a soil extract nutrient were used in topical experiments. Radiolabeled GB was used in these experiments. Individual plants were contaminated on one stem (branch) or on the leaves on one stem. Plants were harvested at 30 minutes, 1, 2, 4, 6, and 24

hours after application of 1 μL of agent (the 30 minute sample was used in place of a zero time sample because this was the shortest time in which the plant could be contaminated, harvested, sectioned, ground, or cut, and extracted). It was found that considerable agent was lost from the plant during the first 30 minutes. Approximately 1,000 μg of agent were placed on the individual plants at the specified contamination site. Some of the agent soaked rapidly into the woody branch, the rest evaporated. Only 350 to 520 μg was recovered 30 minutes later. The agent was rapidly lost from the plants, most being gone in 6 hours. Only a very small quantity of agent was found after 24 hours. The agent stayed mostly at the contamination site but apparently a small amount of agent did move from the point of contamination to other plant parts. The results are shown in Figures 25 and 26. The abbreviation (P.O.A.) is used in Figure 26 for the stem plot. This means point of application.

After the agent had disappeared from the plants, radioautographs were prepared using stem, leaves, and root sections. These autographs revealed considerable radioactivity was distributed throughout the plant even though the agent was gone or at very low levels. Liquid scintillation analysis of these extracts also revealed the same thing. As a result, the extracts were propylated and analyzed by GC. The compound was identified as IMP by GC retention time and thin layer chromatography. IMP is a hydrolytic decomposition product of GB. The results are shown in Figures 27 and 28.

Compared to Figures 25 and 26 the results shown in Figures 27 and 28 indicate that as the agent is lost the concentration of IMP increased. The results also indicate that the IMP readily distributes throughout the plant, even to the roots of the stem contaminated plants. It is not completely clear if the IMP was formed after the agent had moved into the respective plant parts and then hydrolyzed or if the IMP formed at the application site and then translocated to the other plant parts. It is surmised that although a small amount of agent moved from the leaves or stem or lower plant segments and subsequently could have hydrolyzed, most of the IMP was probably formed at the application site and then translocated to other parts of the plant. This supposition is further strengthened by the finding that the weight of IMP in the root started increasing (4 hours) after most of the agent applied to the stem had disappeared or had hydrolyzed to IMP (see Figure 28). No agent was ever found in the roots of topically contaminated plants. The IMP content continued to increase in the roots to a level at 24 hours that far exceeded the total agent content remaining in the plant after 6 hours. Approximately 50 μg GB and 50 μg IMP were found in the total plant at 6 hours. At 24 hours 15 μg GB and 225 μg IMP were found. Apparently IMP can move up to the aerial portions of the plant and down into the roots.

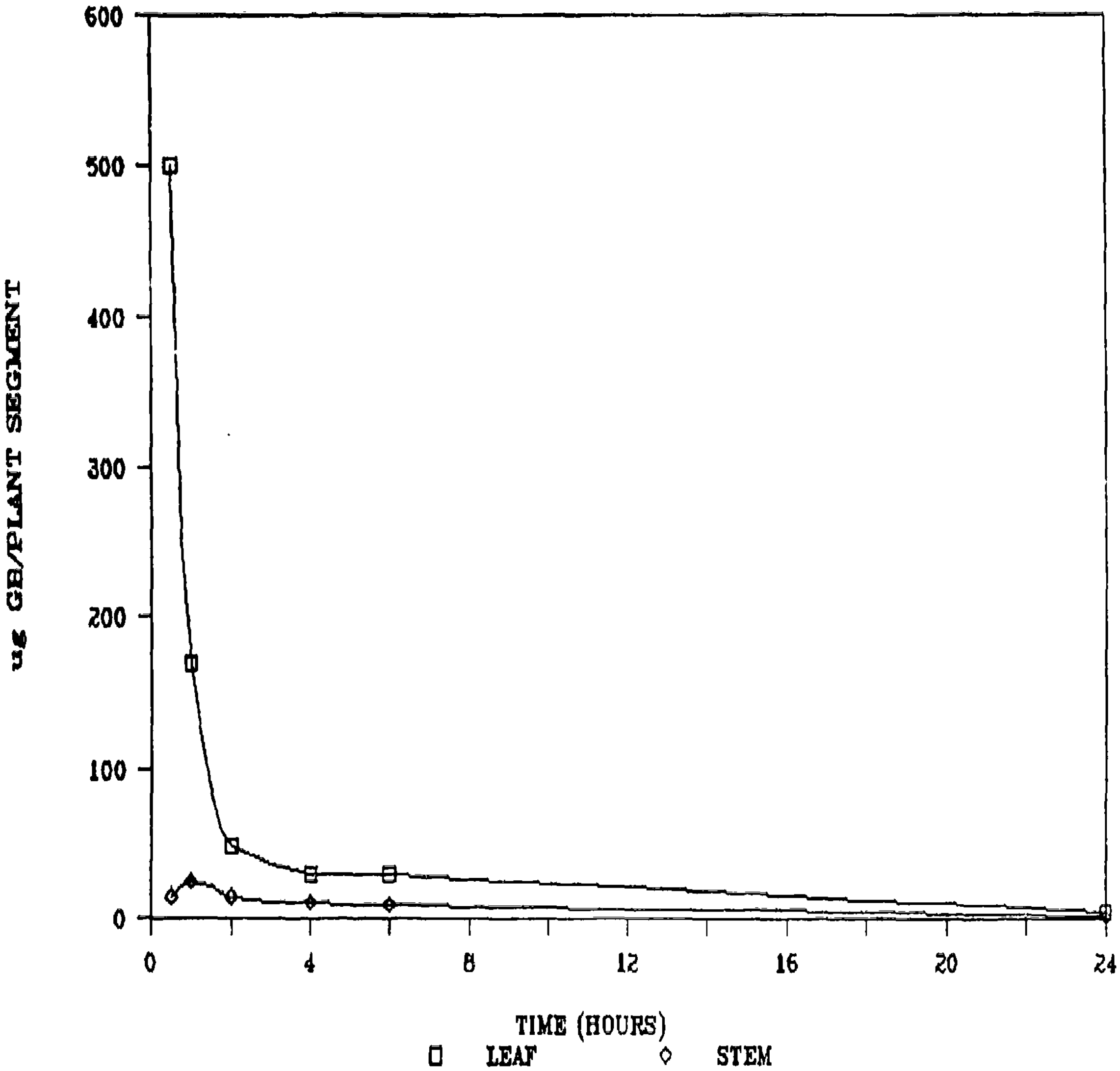


Figure 25. Elimination of GB by Budsage After Topical Application of GB to a Leaf.

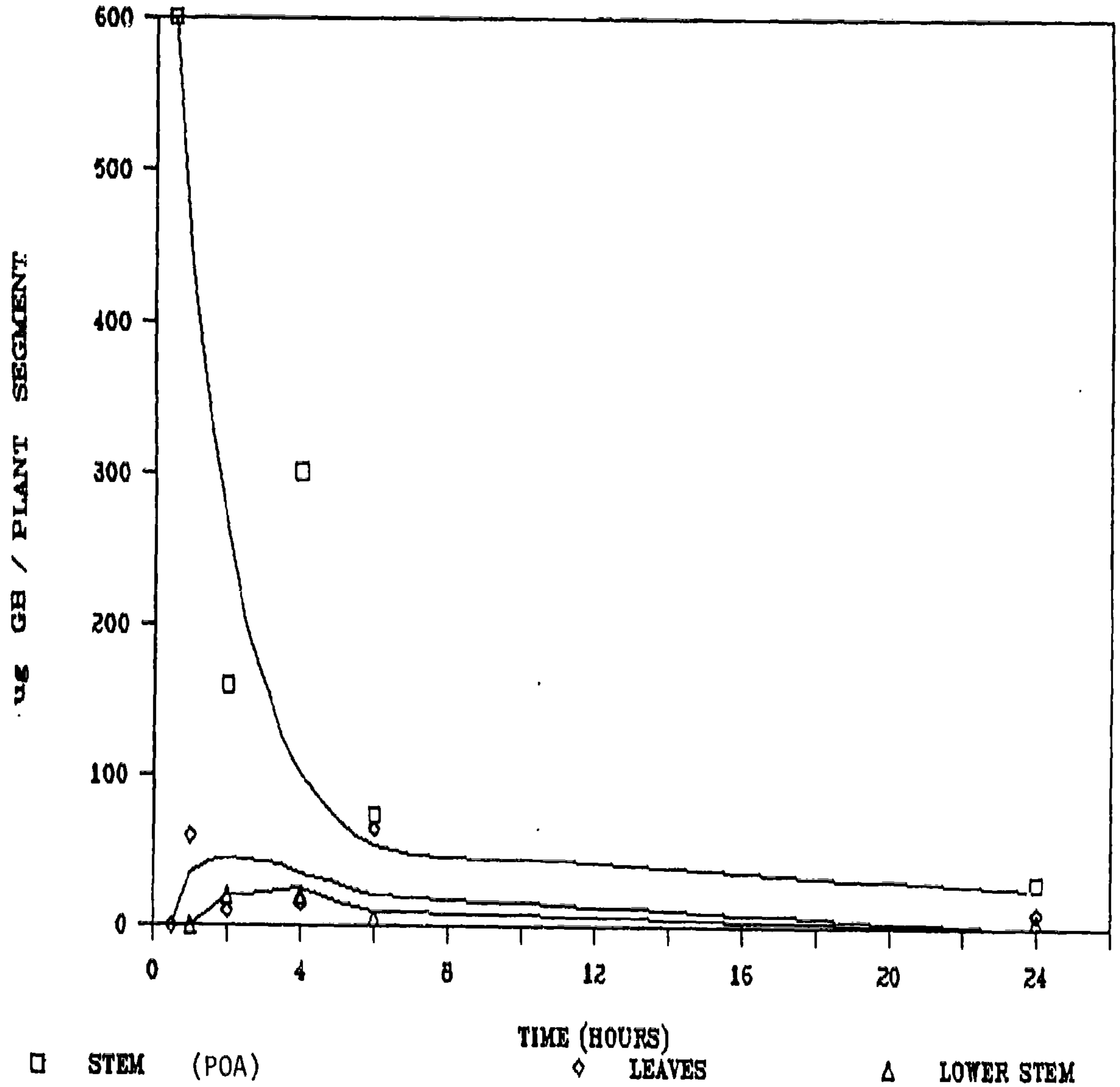


Figure 26. Elimination of GB by Budsage After Topical Application of GB to a Stem.

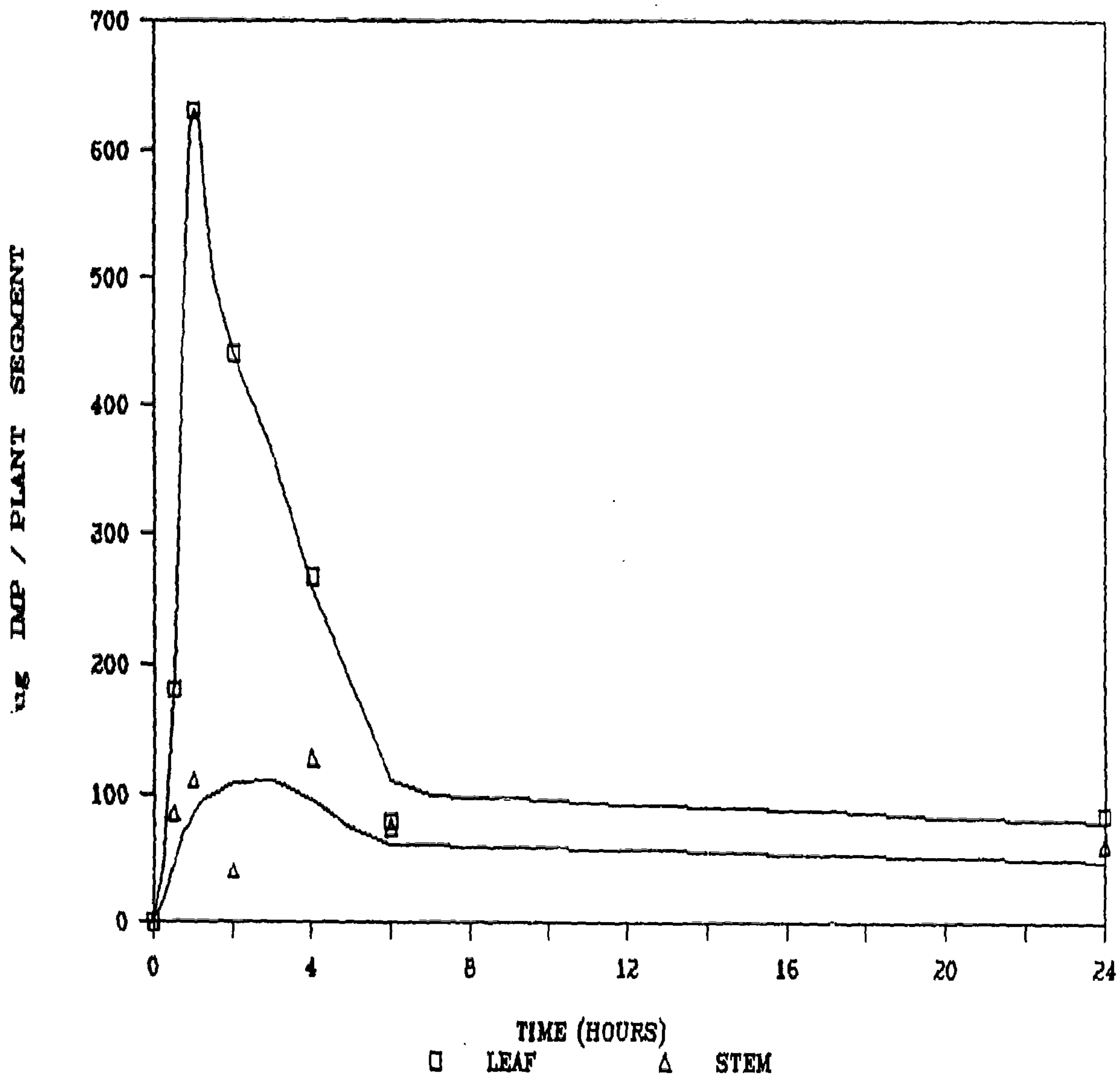


Figure 27. Accumulation of IMP in Plant Segment After Topical Application of GB to Budsage Leaves.

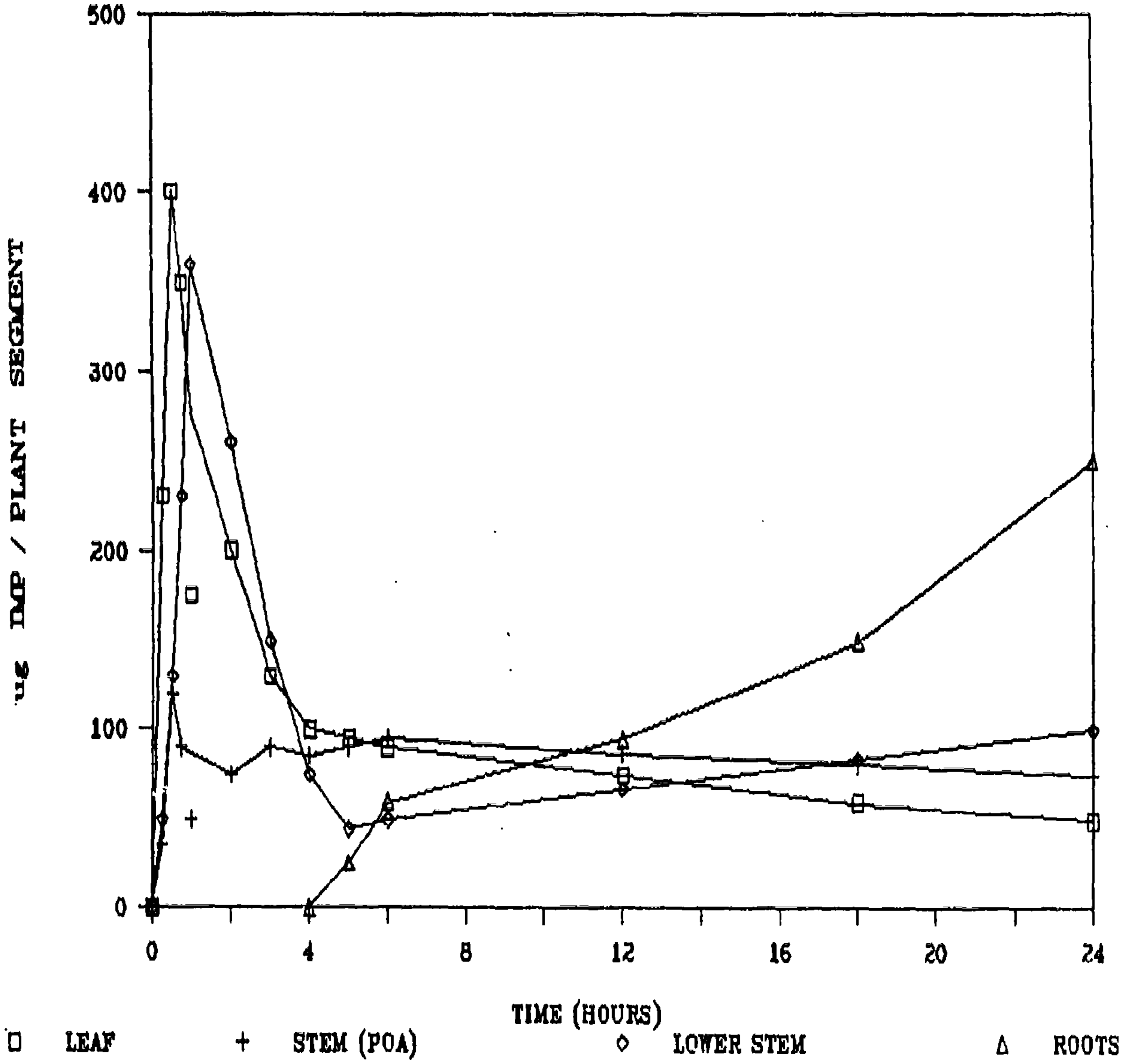


Figure 28. Accumulation of IMP in Plant Segments After Topical Application of GB to a Budsage Stem.

Topical contamination studies also were conducted using cheat grass. The agent was applied individually to the seeds, upper most leaf, or the stem below the leaves. As was observed with the budsage, a large portion of the GB disappeared from the plant during the first 30 minutes after contamination. The agent did not soak as readily into the cheat grass plant parts as was observed to occur with the budsage. Thus, most of the applied agent was lost through evaporation. The agent that penetrated the application sites was rapidly lost from the plants. All agent was gone from the cheat grass plants in 6 hours. The results are shown in Figures 29 thru 31. The abbreviations AL and BL are used to plot the stem data in Figure 30. These mean above leaf and below leaf.

A control plant (uncontaminated plant) was processed in the same manner as the agent contaminated plants. The results obtained from the agent contaminated plants were compared to the results obtained from the control plant. As would be expected no agent or agent related decomposition products were found in the control plant samples.

(2) Uptake Studies

Budsage plants were grown in a soil extract nutrient solution that was contaminated with GB to yield a concentration of 225 μg GB/mL of nutrient. The results are shown in Figure 32.

The agent was rapidly taken up by the budsage plants and translocated into the stem. The highest concentration of agent was found in the stem followed by the roots. No agent was found in the leaves at any of the sampling periods. This indicates either the agent was rapidly decomposed in the stem before the agent could reach the leaves or the agent was rapidly decomposed after reaching the leaves.

To investigate the mode of GB loss from the budsage plants, the samples that were prepared during the agent uptake study were propylated and analyzed for agent related decomposition products. As was found in the budsage topical contamination studies, IMP was found in the root, stem, and leaf samples. After 1 hour exposure of the budsage plants to agent contaminated nutrient, a very large amount of IMP (2,400 μg total) was found in the stems (branches). Much smaller amounts (100 to 200 μg total) were found in the roots and leaves. In 4 hours the amount of IMP stayed nearly the same in the roots but was over 1,600 μg in the leaves. The results are shown in Figure 33.

The information contained in Figure 33 indicates the agent is rapidly decomposed through hydrolysis after the agent is taken up by the roots. The hydrolysis of GB in the soil nutrient

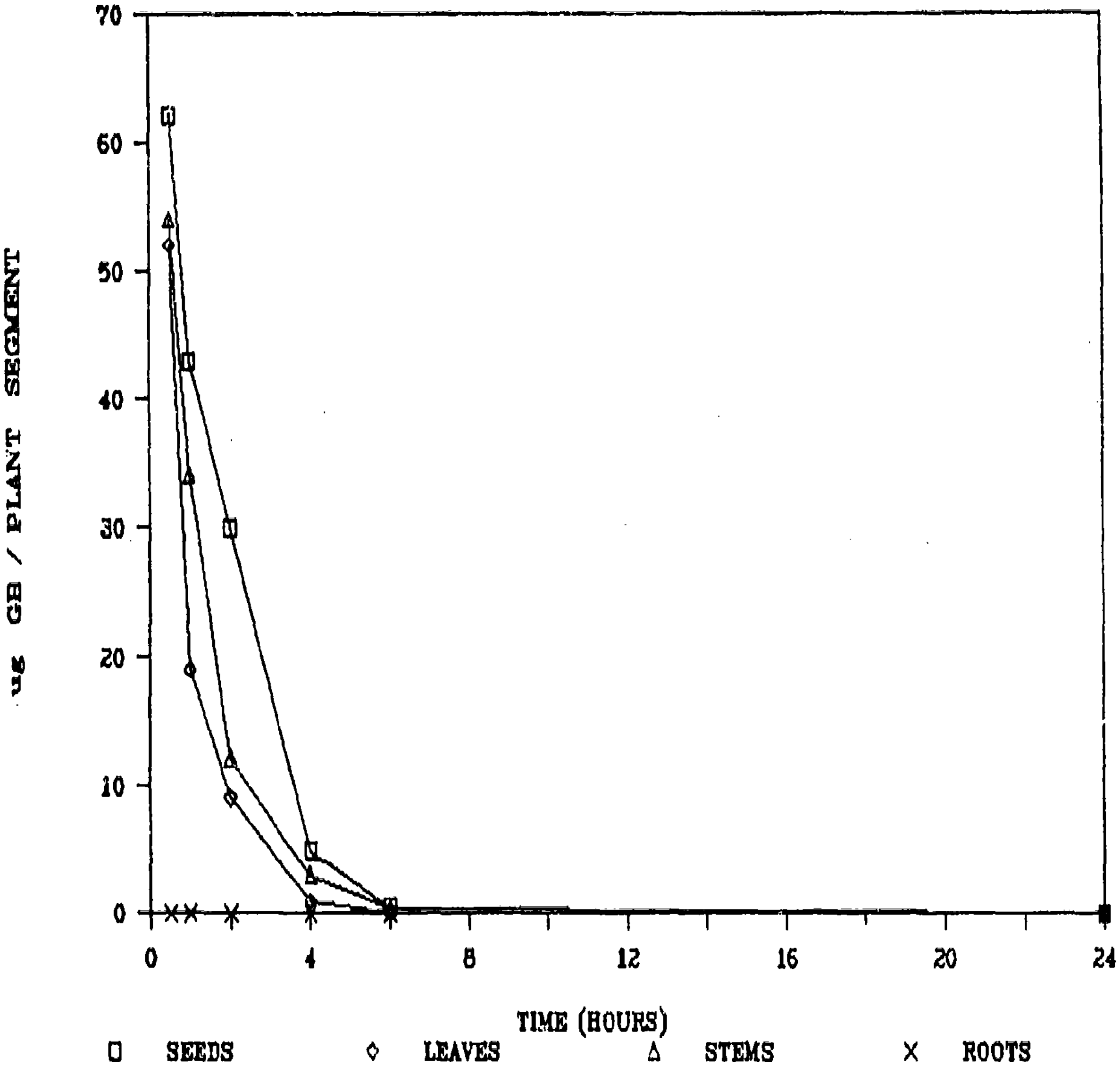


Figure 29. Elimination of GB by Cheatgrass After Topical Application of GB to the Seeds.

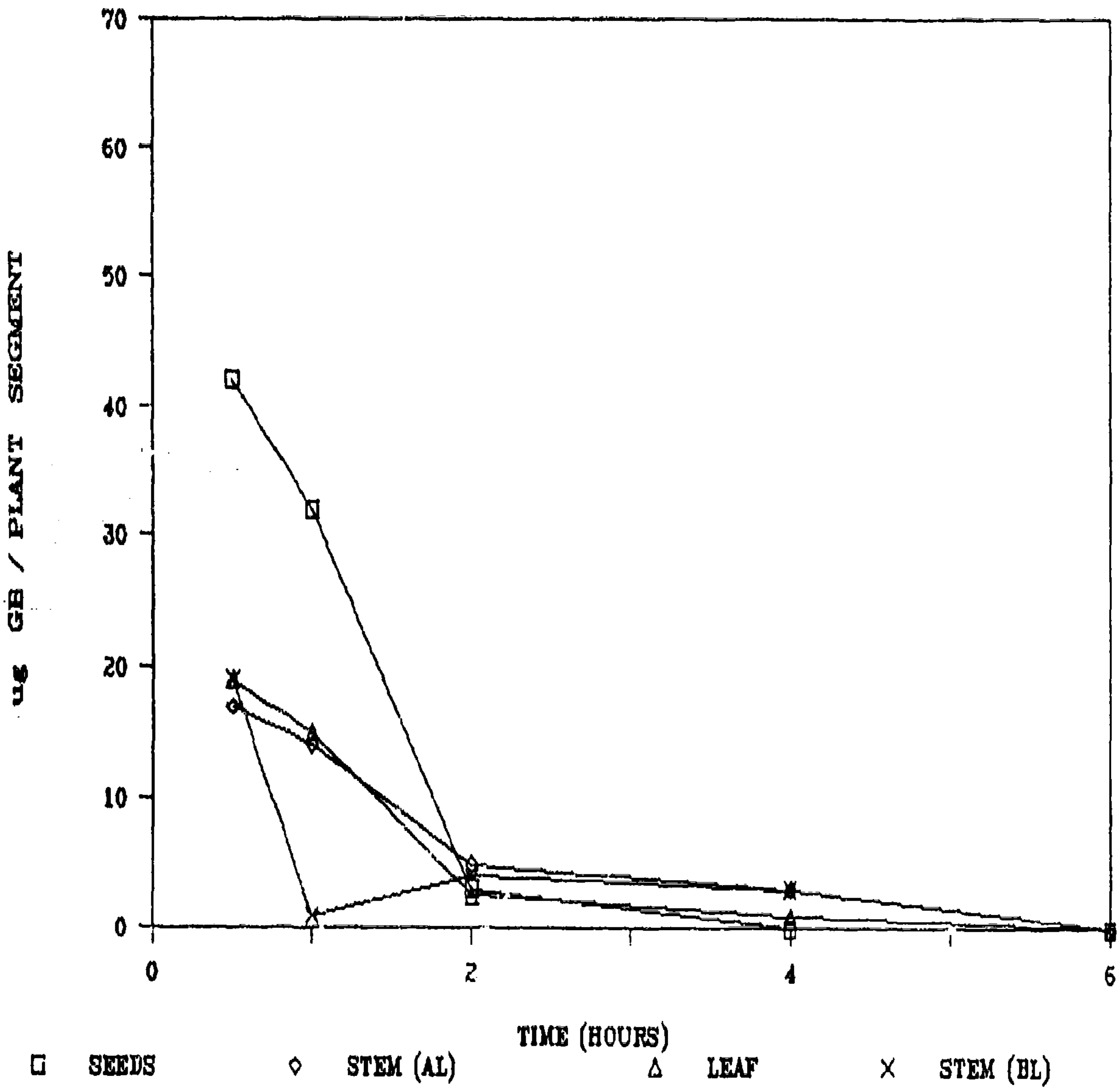


Figure 30. Elimination of GB by Cheatgrass After Topical Application of GB to the Upper Most Leaf.

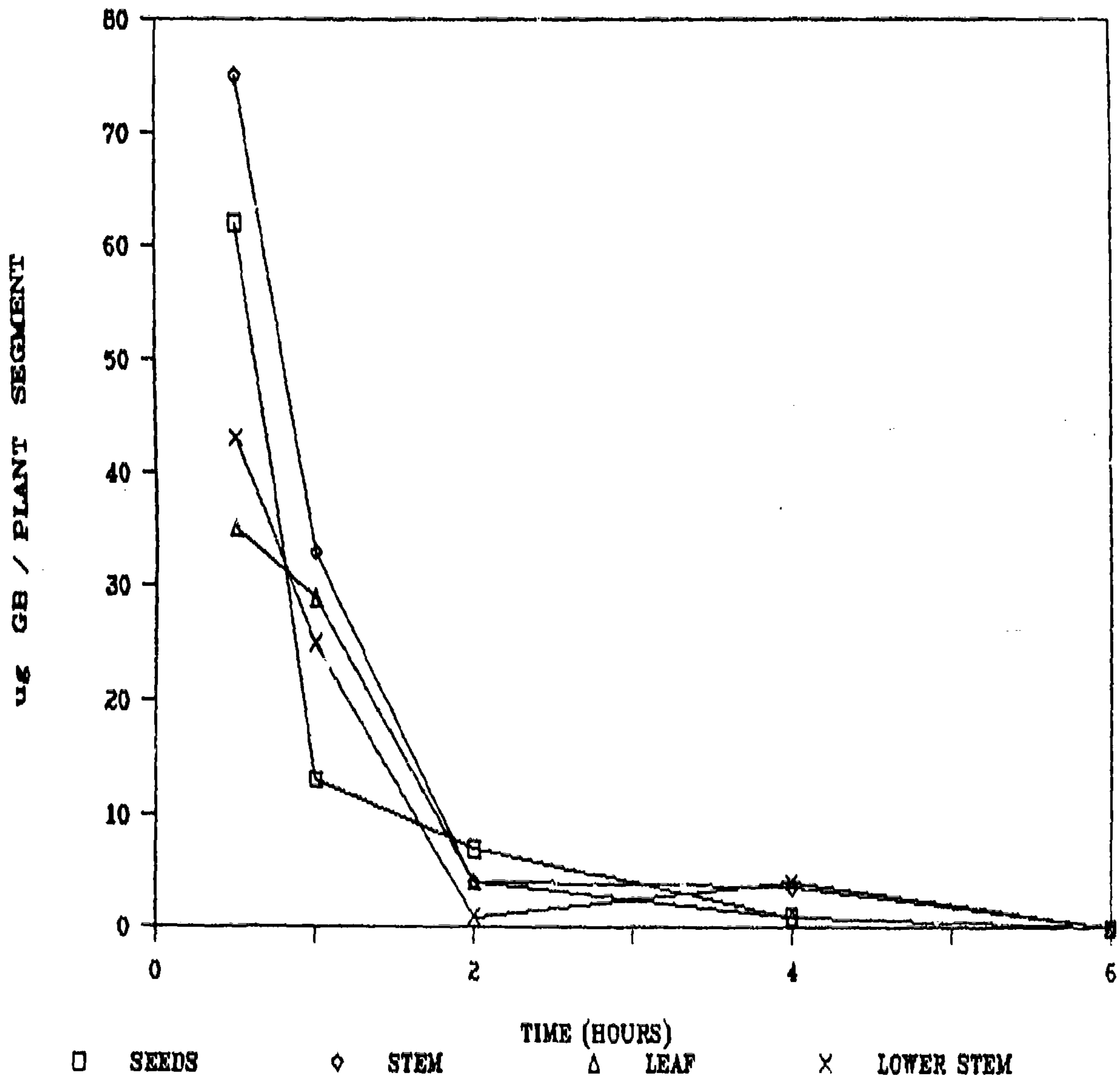


Figure 31. Elimination of GB by Cheatgrass After Topical Application of GB to the Upper Stem.

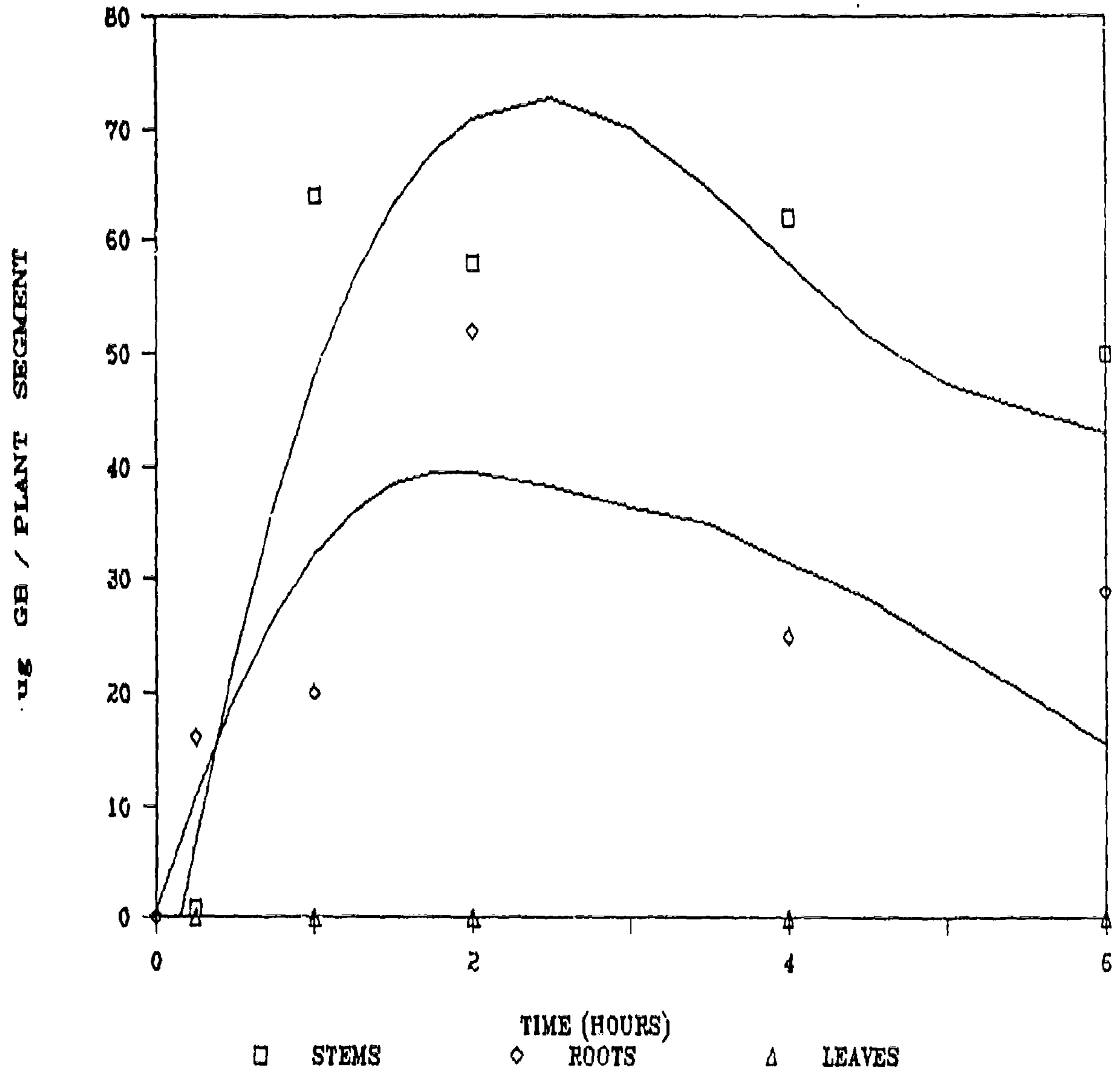


Figure 32. Uptake, Translocation, and Accumulation of GB by Budsage Exposed to 225 µg/mL GB in a Nutrient Solution.

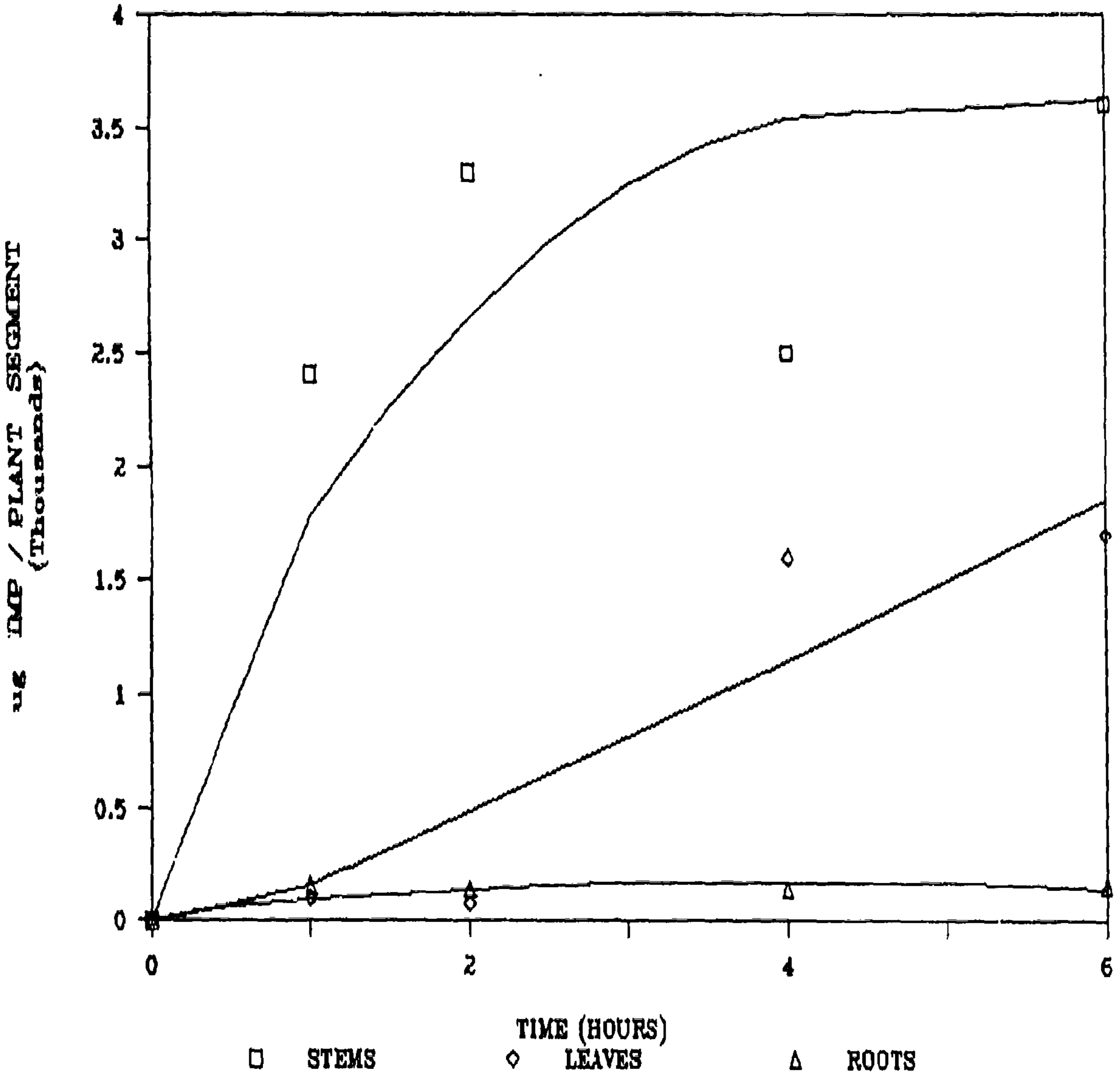


Figure 33. Accumulation of IMP in Plant Segments During the Exposure of Budsage to GB (225 µg/mL nutrient).

was measured at the same time that the uptake studies were conducted. After 2 hours approximately 10 percent of the GB had hydrolyzed in the nutrient and 30 percent had decomposed after 6 hours. This means the concentration of IMP was only 20 μg/mL compared to 200 μg/mL GB in the nutrient after 1 hour (assumingly only IMP is formed in 1 hour by the hydrolysis of GB) and 60 μg IMP/mL in 6 hours compared to 160 μg GB/mL in the nutrient. Thus, the concentration of IMP in the plant samples was unexpectedly high. Reviewing the information contained in Figure 32 shows that the concentration of GB in the roots and stems after one hour were 20 μg and 64 μg/plant segment respectively. The concentration of IMP in the same samples were 200 μg and 2,400 μg/plant segment (see Figure 33). This observation raised the possibility as to the preferential uptake of IMP by the budsage plant as compared to GB. A catalyzed hydrolysis (possibly enzymatic) of the agent when the agent was taken into the plant is another possibility.

As a result of the information obtained in the above experiments, a very brief study of the uptake of IMP from a nutrient solution by bean plants had been made in an earlier study (this study was not repeated with budsage because of the unavailability of budsage plants at the time of the experiment and the time involved in transplanting budsage plants from the desert). It was felt the bean plant studies would give an indication how agent and IMP would be taken up by dicotyledon plants (budsage and beans are both dicotyledons). The results are shown in Figure 34.

The results show that the concentration of IMP in the roots, stem, and cotyledons were 98, 160, and 164 μg/plant segment respectively after 4 days exposure to a 107 μg IMP/mL nutrient. In contrast, the IMP concentration in the budsage plant segments after 4 hour exposure to a 225 μg GB/mL nutrient was 125, 2,500, and 1,625 μg IMP/plant segment. These findings strongly suggest the origin of the IMP in each plant segment is due to the hydrolysis of GB in the plant rather than the preferential uptake of IMP from the nutrient.

The uptake, translocation, and elimination of GB by cheat grass was studied also. The results are shown in Figure 35.

The uptake and translocation of GB by cheat grass is rapid. However, the accumulation of GB is highest in the roots and stems rather than in the leaves as was found in the bean plants. This suggests that the cheat grass has a more efficient means of eliminating GB from the leaves than bean plants. This possibility was not tested. In addition, after 30 minutes there was no trace of agent in any of the cheat grass segments after the plants were transferred to uncontaminated nutrient.

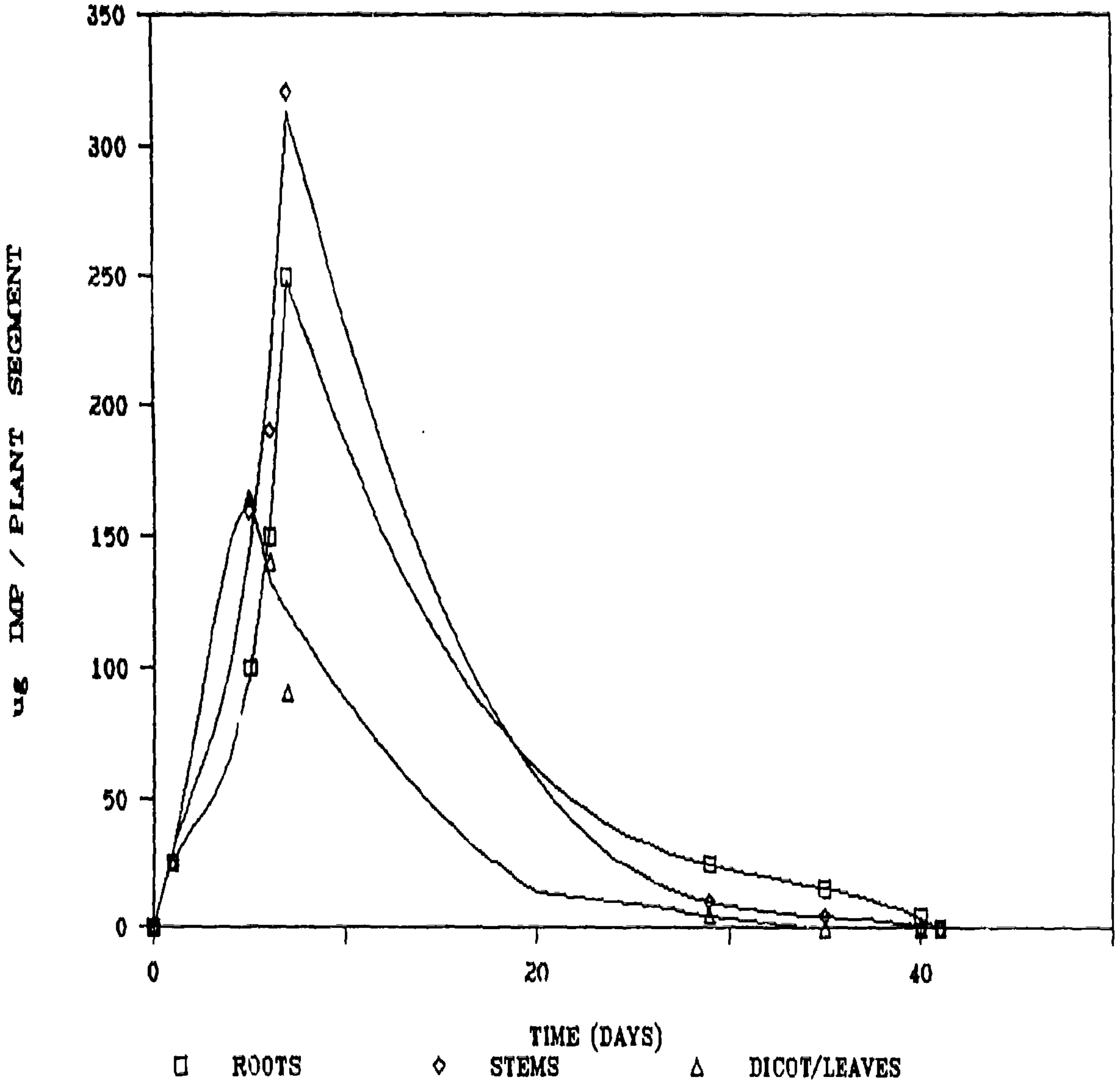


Figure 34. The Uptake, Translocation, and Elimination of IMP by Bean Plants.

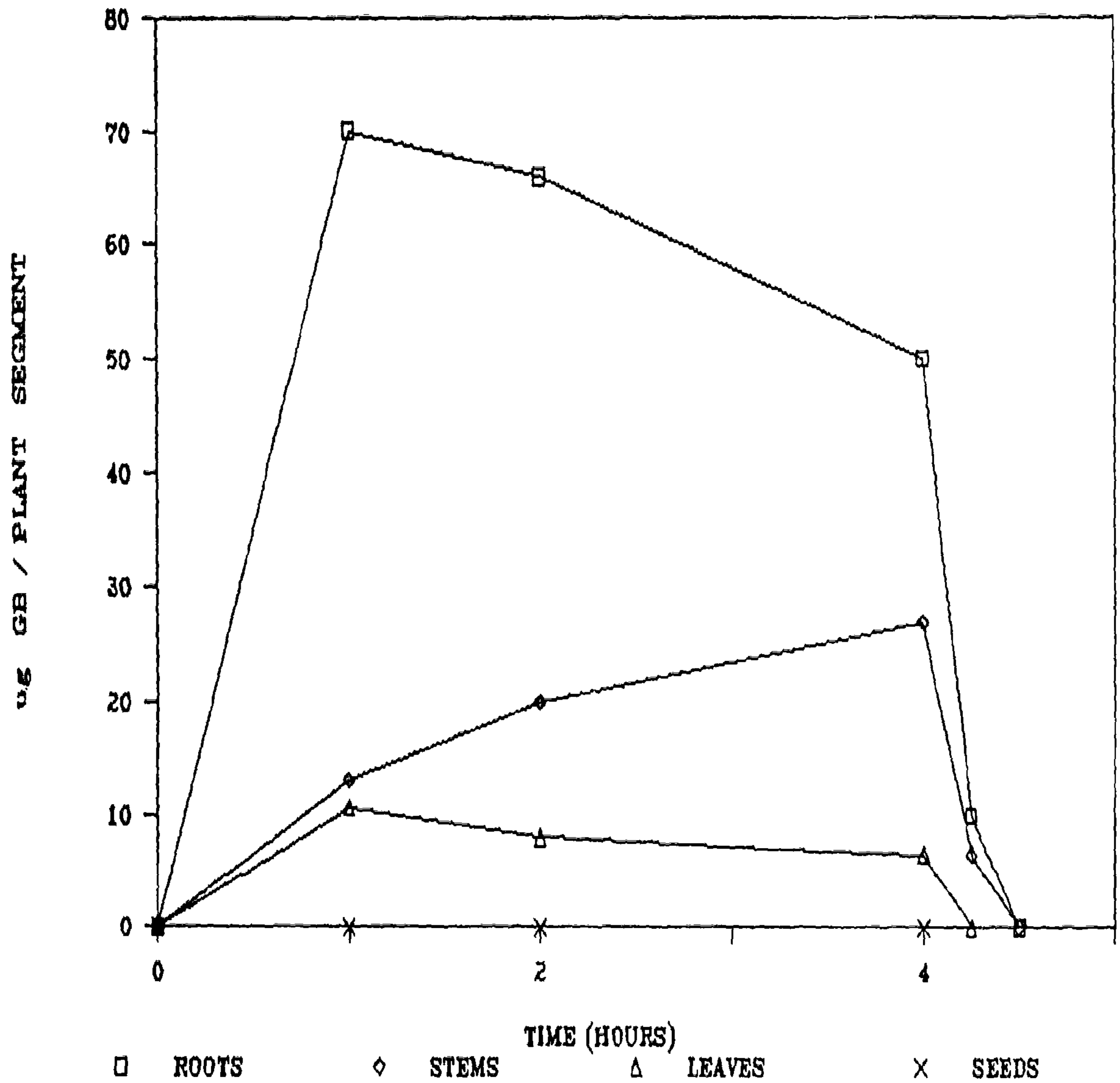


Figure 35. The Uptake. Translocation. Accumulation, and Elimination of GB From a Nutrient Solution by Cheatgrass.

SECTION 3. APPENDICES

A. METHODOLOGY INVESTIGATION PROPOSAL AND DIRECTIVE

METHODOLOGY IMPROVEMENT PROPOSAL October 1982

1. TITLE. Rapid Evaluation of Environmental Hazards (Binary) Improved Binary Agent Persistence and Decay Assessment

2. CATEGORY. Chemical Weapons Testing

3. INSTALLATION. Dugway Proving Ground, Dugway, UT 84022

4. PROJECT OFFICER. Martin J. Houle
Chemical Laboratory Division
STEDP-MT-C-T
AV 789-5225

5. COST. (000)

	FY 83		FY 81 83	
	Required	Funded	Required	Funded
	130		150	20

6. BACKGROUND. Under a prior program to determine the persistence and fate of certain chemical agents, project SAFEST, the loss of GB from soil and dead grass, pickup by living vegetation, and the stability of GB in surface water at different temperatures, were measured versus time. In the experiments with soil and dead grass, the effects of temperature, moisture content, and initial agent concentration were determined by running factorial experiments, performing analyses of variance on the resulting data, and then deriving prediction equations which included the statistical factors and interactions. These equations allow estimating the persistency over a wide range of conditions and were used to prepare multifactor graphs and tables. This work with GB was published as "The Decay of GB in Environmental Samples", September 1972, AD 904-633L.

Sets of more extensive factorial experiments, which included wind speed as well as temperature, moisture content, and initial agent concentration, were run in an environmental chamber with GB, GX, and VX. An initial analysis of the data was performed but funding limitations prevented performing the required analyses of variance, derivation of prediction equations and preparation of multi-factor graphs and tables. Other chemicals of current importance, such as the components and products of binary munitions, have not been investigated.

7. PROBLEM. Before testing of binary chemical systems can be performed data concerning the fate and persistence of the various chemicals is needed for preparing an environmental impact assessment.

8. SOLUTION. This investigation will provide proven experimental methods and data based models for the assessment of the environmental impact caused by binary munition components and products.

9. DESCRIPTION.

a. Available methods and data will be reviewed for applicability to binary systems. For additional required data, chamber testing will be done using factorial experimental design. All data available will be used for predictive model derivation.

b. The data and experimental methods available from prior work with chemical agents will be evaluated for applicability to environmental effects of simulant and agent binary munition testing. Methods and data required for the binary systems will be obtained. Testing will be accomplished in chambers, and the experimental design will be based on expertise obtained in prior similar research. Components and products of binary munition systems will be evaluated for persistency and decay under test environmental conditions. The data obtained will be correlated with prior agent environmental assessments and predictive models will be derived.

c. Outline Plan.

(1) Procure small test chamber.

(2) Review data and literature available.

(3) Design and carry out chamber tests.

(4) Evaluate data and derive predictive models.

d. The investigation will result in procedures for assessing the environmental inpact of testing binary munition systems.

10. END PRODUCT. The end product will be a complete documentation of the fate of G-agents, VX, and binary reaction materials in the DPG environment. In addition, the techniques developed will be applicable to a wide variety of projects.

11. BENEFITS. The Army will benefit from the documentation of agent fate in persistence by being able to prepare necessary environmental documentation related to agent testing.

12. MAJOR ITEMS SUPPORTED.

a. The dissemination of chemicals into the environment requires adequate data for preparing an environmental impact assessment.

b. Dollar Savings. Some of the necessary methods development and experimentation has already been performed at this installation; utilization of prior work and it's publication will eliminate the need for repetition. Likewise, the expertise developed in designing, performing, analyzing, and publishing the prior work will be applied to investigate the environmental effects of additional chemicals in the binary systems.

c. Workload. Conducting field trials is the principal mission of Dugway Proving Ground. A continous effort in this area is in the five year projection.

d. Recommended TRMS priority. #2

13. ECONOMIC ANALYSIS:

a. Financial

(1) Funding Breakdown

	Dollars (Thousands) FY83 IN HOUSE	OUT-OF-HOUSE
Personnel Compensation	62	47
Travel	1	1
Contractual Support	5	-
Consultant & Other Services	-	-
Materials & Supplies	2	2
Equipment	10	-
Subtotals	80	50
FY Totals	130	

(2) Explanation of Cost Categories

(a) Personnel Compensation - These are the wages to be paid the personnel performing the laboratory work, evaluating the data, and writing the report.

(b) Travel - Coordination with TECOM, CSL, and the Contractor.

(c) Contractual Support - Monitoring of Contract.

(d) Consultants or Other Service - None required.

(e) Materials and Supplies - Chemical reagents and miscellaneous glassware.

(f) Equipment - A small environmental chamber is required.

b. Anticipated Delays - None expected.

c. Obligation Plan

Obligation Rate	FQ 1	2	3	4	Total	
In-house	20	25	20	15	80	(Thousands)
Contract	10	15	15	10	50	

d. In-House Personnel. In-house personnel requirements by specialty.

	Number	Manhours Required	Available
Chemist	2	2800	2800
Chem Lab Tech	2	1000	1000
		3800	3800

14. IMPLEMENTATION. The capability to determine the fate and persistence of G-agents, VX, and binary reactants and to prepare the required environmental documentation related to binary testing will be established at DPG. It is projected that this will be accomplished in FY84. Once the necessary test data is obtained from the laboratory investigations the preparation of the environmental documentation can be implemented either by contact or in-house.

15. MILESTONES.

	BFY
	O N D J F M A M J J A S
In-house	- - - - - - - - - - - R
Contract	- - - - - - - - - - - R

16. ROADMAP. See 13c and 15.

17. COST SAVINGS. It is difficult to calculate acutal costs savings resulting from this investigation. However, many intangible savings may result because the environmental documentation and associated data is available to the Army thereby eliminating delays in testing because of concerns expressed by government agencies, concerned organizations and the general public.

18. ASSOCIATION WITH TOP PROGRAM. None

19. AUTHENTICATION.

METHODOLOGY IMPROVEMENT PROSPOSAL December 1983

1. TITLE. The Effect of Chemical Agents on the Environment

2. CATEGORY. NBC Survivability, both Effect and Protection, and Munitions

3. INSTALLATION. Dugway Proving Ground, Dugway, Utah 84022

4. PRINCIPAL INVESTIGATOR. Dr. Frank D. Bagley
Chemical Laboratory Division
STEDP-MT-C-T
Autovon 789-5225

5. PROBLEM. The testing of binary chemical systems cannot be performed until data needed to prepare environmental assessment documentation concerning the fate and persistence of binary associated chemicals in the DPG environment is obtained.

6. BACKGROUND: Extensive studies concerning the persistence and fate of chemical agents in the environment have been conducted in prior years. In addition we have tried to establish the impact caused by the dissemination of binary munition reactants. These investigations have been conducted under the MMT methodology program, TRMS No. 7-CO-PB1-DP1-004, and the in-house laboratory independent research, TRMS No. 7-CO-IL8-DP1-001. Part of the information obtained was from these two investigations was published in the report entitled "The Decay of GB in Environmental Samples," DTC 73-852, September 1972, AD 904 633L, and "Laboratory Comparison of the Persistence of EA 1356 and GB in Soil and Vegetation (U)," DPGTR-C435A (CONFIDENTIAL), September 1983, AD C032590 L However, considerable information concerning the fate and persistence of GB, VX, and QL remains to be analyzed and published in a comprehensive final report. Also additional laboratory studies as to the fate and persistence of QL and DF are required before the environmental effects of binary testing at DPG can be completed.

7. GOAL. To provide proven experimental methods and data based models for the assessment of the environmental impact caused by agents, binary munition components and reaction products.

8. DESCRIPTION OF INVESTIGATION.

a. Available methods and data will be evaluated for applicability to binary systems testing. For additional required data laboratory testing will be done using factorial experiment design. All data available and obtained from this investigation will be used for predictive model derivation.

b. The U.S. Army Dugway Proving Ground will review and evaluate all data and experimental methods obtained from prior work with chemical agents and binary components, for applicability to predicting the environmental effects of binary agent munition testing. Additional methods and data required for the binary systems will be developed. The testing will be accomplished in the laboratory and the experimental design will be based upon the expertise obtained in similar prior research. Components and products of binary munition systems will be tested for persistency and decay under test environmental conditions. The data obtained will be correlated with prior agent environmental assessments and predictive models will be derived.

9. JUSTIFICATION.

a. Associated with Mission. The determination of the fate and persistence of G-agents, VX, and binary munition components is necessary in order to prepare the necessary environmental documentation related to binary munition testing. Once the necessary test data is obtained from the laboratory investigation the environmental documentation can be prepared either in-house or by contract.

b. Present Capability, Limitations, Improvements, and Impact of Test if not Approved. DPG presently has the expertise to conduct the necessary experiments need to obtain the type of data needed to prepare required environmental documentation. If the investigation is not conducted critically needed data will not be obtained and binary munition testing may be delayed.

c. Dollar Savings. It is difficult to calculate actual cost savings resulting from this investigation. However, intangible savings may result because the environmental documentation and associated data will be available to the Army thereby eliminating delays in testing because of concerns expressed by government agencies, other concerned organizations and the general public.

d. Workload. Conducting field and chamber trials and environmental studies is the principal mission of DPG. A continuous effort in this area is projected for the next five. This investigation will be given priority consideration.

The Effect of Chemical Agents on the Environment Continued

10. RESOURCES.

a. Financial.

	Dollars (Thousands) FY 84 In-House	FY 85 In-House
Personnel Compensation	17	49
Travel	1	2
Materials and Supplies	2	4
FY Totals	20	55

b. Explanation of Cost Catagories

(1) Personnel Compensation. This represents the compensation chargeable to the investigation for using technical and other civilian personnel assigned to the investigation.

(2) Travel. Coordination with CRDC and other interested agencies.

(3) Material and Supplies. Chemical reagents, animals, and miscellaneous glassware.

c. Obligation Plan.

FQ	1	2	3	4	Total
Obligation Rate (Thousands)	0	4	12	4	20

d. In-House Personnel. In-house personnel by specialty.

	Number	Manhours FY 84 Required	Available	Total
Chemist, GS-1320	1	540	540	2320
Biochemist, GS-1320	1	100	100	250
Microbiologist, GS-0403	2	100	100	300
Toxicologist (Military)	1	100	100	300
Chem Lab. Tech. (Military)	1	500	500	1200
		1340	1340	4370

11. INVESTIGATION SCHEDULE.

	FY 84												FY 85											
	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
In-House	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R

12. ASSOCIATION WITH TOPS PROGRAM. None

13. AUTHENTICATION.

DEPARTMENT OF THE ARMY Mr. Deaver/ecw/AUTOVON
HEADQUARTERS, U.S. ARMY TEST AND EVALUATION COMMAND 283-2375
ABERDEEN PROVING GROUND, MARYLAND 21005

REPLY TO
ATTENTION OF

DRSTE-AD-M 22 JUN 1981

SUBJECT: Directive, Rapid Evaluation of Environmental Hazards (Binary) Improved Binary Agent Persistence and Decay, TRMS No. 7-CO-PB1-DP1-004

Commander
US Army Dugway Proving Ground
ATTN: STEDP-PO-E
Dugway, Utah 84022

1. Reference TECOM Regulation 70-12, TECOM Methodology Investigation, 1 June 1973.

2. This letter and attached STE Forms 1188 and 1189 (Inclosure 1) constitute a directive for the subject investigation under the TECOM Methodology Improvement Program BP5397-5071.

3. The information at Inclosure 2 is the basis for headquarters approval of the special investigation.

4. Special instructions:

a. All reporting will be in consonance with paragraph 9 of the reference. The final report, when applicable, will be submitted to this headquarters, ATTN: DRSTE-AD-M, in consonance with Test Event 53, STE Form 1189.

b. Recommendations of new TOP's or revisions to existing TOP's will be included as part of the recommendation section of the final report. Final decision on the scope of the TOP effort will be made by this headquarters as part of the report approval process.

c. The addressee will determine whether any classified information is involved and will assure that proper security measures are taken, when appropriate.

d. Upon receipt of this directive, test milestone schedules will be immediately reviewed in light of known other workload and projected available resources in accordance with provisions of paragraph 1-3 to TECOM Regulation 70-8. If rescheduling is necessary, this headquarters, ATTN: DRSTE-TO-O and DRSTE-AD-M will be notified by 1st Indorsement not later than 14 July 1981. If schedules can be met, a P-8 entry will be made directly into the TRMS master file by that date.

e. The Methodology Improvement Division point of contact is Mr. William H. Deaver, ATTN: DRSTE-AD-M, AUTOVON 283-3677/2170.

FOR THE COMMANDER:

Grover H. Shelton

2 Incl
as

GROVER H. SHELTON
C, Meth Imprv Div
Analysis Directorate

APPENDIX B. CHEMICAL METHODS

B. CHEMICAL METHODS

B.1 EXTRACTION PROCEDURES

B.1.1 SOILS

B.1.1.1 Extraction of VX from Soil

A. Procedures

(1) High concentration (greater than 1,000 μg/g)

VX was extracted from soil by mixing 30 mL of 2-propanol with 10 grams of soil contained in a 125 mL Erlenmeyer flask. The flask was shaken for 1 hour. An aliquot of the extract was decanted into a 10 mL screw-cap vial and saved for GC analysis.

(2) Low concentrations (less than 1,000 μg/g)

VX-treated soil samples were extracted by placing soil (10 grams) in a separatory funnel. The flask was washed with small aliquots of distilled water (not exceeding a total of 10 mL), which were added to the separatory funnel. The pH was adjusted to 10.5 with NH4OH. To this, 100 mL of chloroform was added. The funnel was shaken for five minutes and the pH of the aqueous phase was adjusted to pH 7 with dilute HCl. The resultant mixture was dripped through Whatman Phase Separation paper. The soil in the separatory funnel and on the filter paper was extracted two additional times. The pooled chloroform fractions were evaporated to incipient dryness on a rotary evaporator. The resultant residue was dissolved in 5 mL of 2-propanol and analyzed by GC.

B. Verification of Extraction Procedures

A series of extractions with 2-propanol was conducted in triplicate at four levels of VX concentration on DPG soil using thirty minutes of shaking on the wrist shaker. The results are

given in Table B-1. The experiment was designed to establish the efficiency of the 2-propanol to extract high concentrations of VX from DPG test area soil and/or define the concentration(s) where the extraction loses efficiency.

Table B-1. Recovery of VX From Shadscale-Gray Molly-Greasewood Soil by 30 Minutes Shaking With 2-Propanol

VX Application Level (µg/g)	Extracted (Percent) Average	Std. Dev.	Coeff. Var.
10,000[a]	71	6	0.085
5,000	72	5	0.069
1,000	9.7	0.6	0.060
100	2.1	0.7	0.003

[a]Recovery of agent using two hour shaking period averaged 87 percent, with s = 4, and v = 0.046.

From this work, it was concluded that some VX is held strongly on soil binding sites, such that concentrations below 0.5 percent are not adequately extracted by alcohol.

The procedure for extracting low concentrations of VX from soil was shown to have improved recovery. Applying this method to initial concentrations of 0.1 and 1.0 µg VX/g of air-dried and moisture adjusted DPG surface soil (approximately one percent soil-moisture content and five percent soil-moisture content) yielded the results shown in Table B-2.

Table B-2. Recovery of Low Levels of VX From DPG Soil (Percent Recovered)

	Initial Concentration			
	0.1 µg/g		1.0 µg/g	
Days	1% H20	5% H20	1% H20	5% H20
0	86	105	99	82

B.1.1.2 Extraction of GB from Soil

A. Procedure

Ten grams of soil contained in a 125 mL Erlenmeyer flask were mixed with 30 mL 2-propanol. The mixture was shaken vigorously for one hour on a wrist action shaker. An aliquot of the liquid extract was decanted and filtered into a 10 mL screw-cap vial through a millipore filter when the sample had suspended soil particles or vegetative matter and saved for gas chromatographic analysis.

B. Verification of Extraction Procedure

Greater than 95 percent of the GB originally applied to oven dried soil was extracted at "zero-time". This high efficiency of agent recovery demonstrates the reliability of the 2-propanol solvent extraction method. It was found that the amount of GB recoverable from samples of oven-dried soil contained in a closed flask remained high for 6 days so long as the sample was protected from moisture. Table B-3 shows the recovery of GB from oven-dried soil (collected from the shadscale-gray molly-greasewood area at DPG) at various time intervals after agent application and is representative of the extraction efficiencies obtained throughout this investigation.

The small observed loss of GB was probably due to hydrolysis resulting from the small amounts of moisture introduced at the time of agent application and to a small evaporative loss.

The effect of 2-propanol upon the decay of GB in soil was investigated. This was necessary because standard solutions of GB in 2-propanol were used to apply a specific weight of GB to some samples and it was felt the solvent might change the rate at which GB disappears from the soil. The results were compared to those obtained from a study in which undiluted GB droplets were added to soil. It was found that there was no significant difference in the decay rate of GB when added in 2-propanol or as undiluted agent in closed flask experiments. However, in experiments in which GB in 2-propanol was added to soil contained in petri dishes, 1g of soil was placed as a thin film across the bottom of the petri dish and exposed to a gentle airstream (2-4 mph) 45 percent of the GB was lost while the 2-propanol was evaporating from the dish (usually the 2-propanol evaporated in 5 to 10 minutes). The values obtained from samples treated in this

manner were corrected for this evaporative loss. (In comparison, experiments in which undiluted GB was added to thin films of soil and exposed to a gentle airstream showed a 30 to 40 percent loss in 10 to 15 minutes).

Table B-3. Recovery of GB From Oven-Dried Shadscale-Gray Molly-Greasewood Soil[a]

Sampling Time	Agent Application Level (μg/g Soil)	Agent Extracted (μg/g Soil)	Percent Recovered
1 hour	1000	975	97.5
4 hours	1000	1000	100.0
8 hours	1000	930	93.0
12 hours	1000	880	88.0
1 day	1000	825	82.5
2 days	1000	795	79.5
4 days	1000	775	77.5
6 days	1000	780	78.0

[a]The samples were stored at 20 °C.

B.1.2 VEGETATION

B.1.2.1 Extraction of VX from Vegetation

A. Procedure

Fifty grams of ground dried vegetation was added in small portions to a 1 1/2 inch x 12 inch extraction column (a small piece of methanol washed glass wool was placed in the bottom of the column). The column was connected to vacuum and tamped slightly with a rod between additions. A filter paper circle was placed on top of the column.

The column was extracted with acidic methanol (0.1N HCl) prepared by adding 8.2 mL concentrated HCl to one liter of analytical grade absolute methanol. The alcohol addition rate

was adjusted with the stopcock at the bottom of the extraction column so that the extraction with one liter of solvent required 2 to 3 hours.

The acidic methanol was evaporated under vacuum on a rotary evaporator at a temperature of 50 to 60 °C. The residue was dissolved in 100 mL of 0.1 N HCl in methanol, which was then shaken with 100 mL analytical grade chloroform. The organic layer was separated from the aqueous phase and the HCl solution was extracted three more times with 100 mL portions of chloroform. The chloroform extracts were combined. The aqueous layer was made basic (pH 10.5) by portionwise addition of potassium carbonate or potassium bicarbonate in the presence of 100 mL of chloroform. The separatory funnel was shaken and cautiously vented between additions. Some vegetation samples produced intractable emulsions when the extraction with base was attempted. In these cases, the emulsion was centrifuged and the solvent layers separated from the small amount of solid phase that may be present. The basic aqueous layer was extracted four times with 100 mL portions of chloroform and the extracts combined. Each combined chloroform extract (acidic and basic) was evaporated to incipient dryness under vacuum at a temperature of 50 °C and the residue dissolved with a minimum, measured quantity (usually 1 mL) of spectrograde 2-propanol or absolute ethanol and stored in a refrigerator until analyzed.

B. Verification of Extraction Procedure

The efficiency of the acidic methanol method, which was determined using spiked samples, is shown in Table B-4. The precision of the extraction is shown in Table B-5.

Table B-4. Efficiency of The Acidic Methanol Column Extraction Procedure

Sample	VX[a] added to Column, μg	VX Recovery by GC, μg	Percent Recovery
1	0	0	--
2	0	0	--
3	5	4.6	92
4	5	4.8	96
5	25	25.2	100.8
6	25	24.6	98.4

[a]VX in absolute ethanol was pipetted onto the top of column just prior to elution with acidic methanol.

Table B-5. Precision of The Acidic Methanol Column Extraction Procedure

Sample	VX Concentration by GC, $\mu g/Kg$
1	104
2	98
3	132
	x = 111
	s = 18

In another check on extraction efficiency, samples of dried cheat grass were contaminated with known amounts of VX and stored, one set at 0 °C and one set at 25 °C. At several times after contamination, samples were taken and analyzed using the acidified methanol extraction technique. The results are shown in Table B-6.

The efficiency of the acidified methanol extraction procedure was tested further using P radioisotope labeled VX. Two 50 gram samples of cheat grass were contaminated with 1,900 μg of labeled

VX. One sample was extracted immediately and 97+ percent of the label (assumed to be VX) was recovered. The second sample was extracted 3 days later and 100 percent of the label was extracted. The extracts also were processed through the cleanup procedure and the partitioning of the labeled material between the various solvents was observed and efficiency values for each solvent extraction step was obtained. The sum total of recovered label was 85 percent. Smaller samples (1 g cheat grass) were processed in a similar manner; the extraction efficiency was approximately 100 percent.

Table B-6. Laboratory Study of VX Persistence on Dry Cheat Grass (Acid Methanol Column Extraction Procedure)[a]

Sample Number	Storage Temperature (^{o}C)	Time to Extraction (Days)	VX Recovered ($\mu g/Kg$)	Percent Remaining
1	0	0	25.2	100
2	25	0	24.6	98.4
3	0	3	20.0	80.4
4	25	3	21.1	84.4
5	0	5	21.5	86.0
6	25	5	19.1	76.4
7	0	11	23.8	95.2
8	25	11	21.1	84.4
9	0	50	20.4	81.6
10	25	50	18.2	72.8

[a]VX was added dropwise by micropipetting 0.125 mL of a 200 $\mu g/mL$ solution in ethanol onto 50 grams ground cheatgrass.

The recovery of VX from samples of living and growing vegetation (bean plants, etc.) is quantitative. The procedure to be described for GB (Section B.1.2.2.A) was used for VX contaminated plant material except acidified methanol was used instead of 2-propanol.

B.1.2.2 Extraction of GB from Vegetation

A. Procedure

All samples of dried vegetation (one gram) were extracted by shaking vigorously on a wrist-action shaker for one hour with 30 mL of 2-propanol.

Plant segments of actively growing vegetation were macerated with 10 mL 2-propanol in a tissue homogenizer for 4 to 5 minutes. The mixture was decanted and filtered through filter paper and re-extracted with 5 mL 2-propanol. The two extracts were pooled and analyzed for GB.

B. Verification of extraction procedure

The verification of the GB extraction procedure is discussed in a classified study discussed elsewhere (reference 11).

B.1.3 EXTRACTION OF AGENT DECOMPOSITION PRODUCTS FROM SOIL AND VEGETATION

The extraction of the phosphonic acids YN and IMP from soil and vegetation required a basic aqueous extraction using 1.5N NH_4OH. This extract was then neutralized by the dropwise addition of concentrated HCl and partitioned between chloroform and the aqueous phase as previously described (B.1.1.1.A.(2)). The chloroform was evaporated and the residue dissolved in 2-propanol, propylated (using the method in B.2.2.5.B), and analyzed by gas chromatography. The procedure was tested with DPG test area soil. The results obtained are shown in Table B.7.

Table B.7. Recovery of YN and IMP From Soil

Application Level		Average Percent Recovered[a]
YN	10 $\mu g/g$ soil	66.3
	100 $\mu g/g$ soil	83.3
	1,000 $\mu g/g$ soil	83.3
IMP	10 $\mu g/g$ soil	51
	100 $\mu g/g$ soil	64
	1,000 $\mu g/g$ soil	80

[a]Triplicate Analysis

The results indicate that the method is satisfactory for identification work and for semi-quantitative analysis. Corrections for unrecovered materials can be made based upon these findings and a reasonable estimate of the original agent concentration can be made.

B.2 ANALYTICAL PROCEDURES

B.2.1 ANALYSIS OF WATER SAMPLES

B.2.1.1 Analysis of VX and Hydrolysis Products in Water

The same general procedure to be described for the analysis of GB in water (B.2.1.2) was used with VX in water. However, water in the alcohol-diluted samples was found to have a deleterious effect upon the GC analysis for VX. As a result, experiments were conducted to study the effect of drying methods and the effect of esterification, before or after drying, on the reproducibility of recovering VX and VX decomposition products from water samples. Table B-8 presents the recovery efficiency achieved upon analyzing water solutions containing 33.3 $\mu g/mL$ each of VX, YL, and YN; these results demonstrate the efficiency of the dilution method for VX and associated decomposition products. The analysis was run in triplicate. In the table, Pr refers to esterification with diazopropane; Na_2SO_4 refers to a sodium sulfate drying method and are shown in the order that they were done.

In a second experiment, triplicate samples of a freshly prepared water solution containing 14 μg of EA 2192/mL, 4 μg of YL/mL, and 7 μg YN/mL were analyzed. The results are shown in Table B-9. The data indicates that esterification followed by drying is essentially quantitative in preparation of aqueous samples of VX, YL, and YN for GC analysis. However, aqueous samples of EA 2192 should be dried prior to esterification (see Table B-9). Other drying techniques, such as the use of calcium chloride and isotropy distillation were tried but, sodium sulfate is the preferred method of drying.

Table B-8. Percent Recovery, Triplicate Samples

Compound	Pr-Na_2SO_4	Na_2SO_4-Pr
VX	99.6	105.2
YL	96.4	85.7
YN	99.5	81.8

Table B-9. Percent Recovery, Triplicate Samples

Compound	Pr-Na_2SO_4	Na_2SO_4-Pr
2192	84.5	102.7
YL	103.8	100.0
YN	101.1	87.8

B.2.1.2 Analysis of GB and Hydrolysis Products in Water

Portions of each surface water sample were treated with sufficient undiluted GB to produce agent concentrations of approximately 10 μg and 100 μg GB/mL water. Aliquots (one mL)

were withdrawn immediately to establish the initial GB concentrations. These 1 mL aliquots were diluted with 9 mL of 2-propanol, thoroughly mixed, and analyzed for GB by GC. The recovery of agent was quantitative at zero time.

The method of water sample analysis was tested using a simulated GB hydrolysate. A water solution containing 10 µg/mL GB, 7 µg/mL IMP and 2 µg/mL YN was prepared. An aliquot was analyzed for GB. Another aliquot was analyzed for YN, and IMP following esterification with diazopropane. The results are shown in Table B-10.

Table B-10. Recovery of GB and Associated Decomposition Products From Simulated GB Hydrolysate

	µg/mL Initial	µg/mL Recovered	Percent Recovered
GB	10	8.3	83
YN	2	1.4	70
IMP	7	7	100

B.2.2 GAS CHROMATOGRAPHY

Because of the chemical complexity of many of the extracts from environmental samples, classical chemical analysis generally is unsatisfactory without extensive clean-up of the extracts prior to analysis. This is both time-consuming and a possible source of serious analytical error due to loss of the compounds of interest during clean-up and/or separation from extraneous materials present in the extracts. GC analysis provides the needed separation so it has been extensively used at DPG for the analysis of agents and simulants in test samples (references 12-14).

B.2.2.1 Column Preparation

The acidic sites generally present on the surface of the glass columns are removed by treating the columns with' dimethyldichlorosilane (DMCS) prior to packing. The empty column was filled with a 15 percent (v/v) solution of DMCS in toluene and allowed to stand for 10 minutes at room temperature. The solution was then removed and the column thoroughly rinsed with several 50 mL portions of toluene and finally with methanol. The final rinse by methanol was continued until the methanol was neutral to pH paper. Stainless steel columns were also used in initial studies; however, the lower detection limits obtained from the stainless steel columns were not as good as that obtained from the glass columns.

B.2.2.2 Specific Analyses

A number of decomposition products may result from the decomposition of VX in environmental samples. However, only four were found in any significant quantity. These are YN, KK, YL, and EA 2192. VX and KK can be chromatographed directly on an Apiezon N column (the retention time of KK is 4.5 times that of VX). EA 2192 can be chromatographed together with VX provided it is alkylated first using the method described in B.2.2.5.B. EA 2192 is of particular interest because of its anticholinesterase activity and high toxicity. This compound has been found in small quantities in alkaline Dugway soils that were purposely treated with VX. The n-propyl or n-butyl esters of of EA 2192 are resolved from VX.

YL and YN cannot be chromatographed directly. These compounds must first be alkylated. It is also best to chromatograph these compounds on a polar column and at lower temperatures than is necessary for the separation and analysis of VX and EA 2192. An Igepal CO-880 column was used for this analysis. However, VX will not elute from this column.

The major decomposition products that arise from GB in environmental samples are YN and IMP. These compounds must also be alkylated prior to GC analysis.

B.2.2.3 Instrumentation

Varian Aerograph Models 1800 and 1520B and Micro-Tek Model MT-220 gas chromatographs were used in various phases of

this investigation. Each GC was equipped with a Flame Photometric Detector (FPD).

B.2.2.4 Column Packing and Operational Parameters

The following three columns were used with the listed settings:

(1) Apiezon N. Column

Column material	glass
Column length	6 feet
Internal diameter	2 millimeters
Column packing	0.2 percent Apiezon N on 80/100 mesh DC-110 glass beads
Carrier gas	Nitrogen
Carrier gas flow rate	40 mL/min
Column temperature	200 ^{o}C
Detector temperature	200 ^{o}C
Injector temperature	200 ^{o}C
Sample size	0.5 μL

(2) Igepal Co-880 Column

Column material	glass
Column length	6 feet
Internal diameter	2 millimeters
Column packing composition	5 percent Igepal CO-880 on 80/100 mesh Gas Chrom Q
Carrier gas	nitrogen
Carrier gas flow rate	30 mL/min
Column temperature	140 ^{o}C
Detector temperature	150 ^{o}C
Injector temperature	150 ^{o}C
Sample size	0.5 μL

(3) OV-225 Column

Column material	glass
Column length	5 feet
Internal diameter	2 millimeters
Column packing composition	5 percent OV-225 on 80/100 mesh Gas Chrom Q
Carrier gas	nitrogen
Carrier gas flow rate	40 mL/min
Column temperature	185 ^{o}C
Detector temperature	200 ^{o}C
Injoctor temperature	200 ^{o}C
Sample size	0.3 μL

B.2.2.5 Derivative Formation

A. Silylation

Unfortunately the high polarity and low volatility of many agent decomposition products renders them unsuitable for direct GC analysis. It is possible, however, to form derivatives of these compounds which are more amenable to analysis by established GC methods. Probably the most widely used technique is that of silylation in which volatile trimethylsilyl derivatives are formed. Although silylation of the non-volatile, acidic agent residues yields derivatives which can be analyzed by GC, three major problems were encountered when this technique was used:

(1) Fogging of optics in flame photometric detectors by SiO_2 formed by the combustion of the silyl compounds.

(2) The appearance of ghost peaks or high positive blanks caused by the reaction of non-volatile sample materials with excess silylating agent remaining in the GC column from previous injections.

(3) The necessity of using only dry and inert solvents in the analysis.

B. Alkylation

An alternative to silylation is the reaction of the acidic decomposition products with diazoalkanes. Although diazoalkanes are toxic and explosive compounds which require considerable handling care, they provide a means by which these organophosphorus acids may be rapidly and quantitatively converted to volatile derivatives at room temperature. In addition, the reaction may be carried out in a variety of solvents.

The procedure followed involves adding excess diazoalkane solution to a measured volume (usually one mL) of an alcoholic solution containing the acidic agent decomposition products (reference 15). Since the esterification reactions are instantaneous and nearly quantitative, persistence of an orange-yellow color in a previously colorless sample signals an excess of diazoalkane and indicates completion of the reaction.

The sample is then reduced to its original volume by gentle heating. The excess diazoalkane is rapidly destroyed during the heating step as indicated by the disappearance of the orange-yellow coloration. The colored samples are analyzed directly for the presence of alkylated residues. Certain complications accompanying the alkylation procedure may require attention when it is applied to materials extracted from environmental sources:

(1) The esterification reaction is nearly quantitative for all protic acids, but salts of these acids are unreactive. For this reason, samples must be adjusted to a pH of less than 7 by adding a drop of 20 percent HCl solution in 2-propanol before adding the diazoalkane. A somewhat larger volume of diazoalkane solution is then required to destroy the small excess of HCl present.

(2) Since the diazoalkanes react indiscriminately with acids, the presence of sulfate and phosphate ions in acidic solutions will lead to the formation of di-alkyl sulfates and tri-alkyl phosphates. While these materials are objectionable due to their relatively high response in the FPD detection system, no satisfactory method has been devised for removing trace amounts of sulfate and phosphate ions from solutions. Direct interference from alkyl-phosphates and sulfates may be avoided in most cases through a judicious choice of alkylating agents. For example, the methyl esters of methyl-phosphonic acid (YN) and phosphoric acid are not adequately resolved by an available GC technique, and therefore, diazomethane cannot be employed for determination of YN in environmental samples. In this case, either diazopropane or diazobutane will give more satisfactory results, since the C3 and C4 alkyl-phosphates and methylphosphonates are easily resolved.

(3) Samples extracted from vegetation usually contain green and yellow plant pigments which mask the characteristic diazoalkane colors that persist when the samples are fully alkylated. In these cases a secondary visual observation, namely, the evolution of nitrogen gas, must be relied upon as an indicator for the esterification reaction. The reaction is complete when further addition of diazoalkane solution fails to bring about a rapid evolution of nitrogen bubbles from the sample.

(4) In order to avoid esterifying acidic non-volatile residues that may be present in the GC column, any excess alkylating agent must be decomposed before injecting a sample into the GC. This is easily accomplished with diazoalkane solutions by gentle heating.

(5) Multiple products are formed from diazopropane and diazobutane because of the formation of isobutyl and isopropyl carbonium ions via a hydride shift. These small peaks do not ordinarily cause any problem but should be taken into account when performing the analysis.

(6) Water and salts in environmental samples have been found to decrease the efficiency of the esterification reaction. It is therefore desirable to use standards which have been made using similar extracted material as the solvent.

B.2.3 THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) has been employed for the qualitative and semi-quantitative identification of VX, GB,and their associated decomposition products. TLC has the advantage over other methods in that it is relatively cheap, micro quantities of material can be detected, it provides a fast method for identification, and, at the same time, gives accurate results.

B.2.3.1 Detection of VX, GB, and EA 2192

For agents VX, GB, and EA 2192 (a compound that exhibits enzymatic inhibitory properties), the lower detection limit is less than 10 nanograms. The developing sprays for these compounds are a beef liver homogenate solution (which contains a cholinesterase) ard an indoxylacetate spray (reference 16). The preparation of these sprays is described below.

A. Preparation of Liver-Homogenate

Livers from 1 to 2 year old steers were used. Livers and glassware were kept in an icebath during the preparation of the

homogenate. The livers were ground in an all-glass homogenizer using 20 g of liver to 180 mL of distilled water. The liver used should be devoid of vascular vessels and membranes. The homogenate was centrifuged at 2000 rpm for 20 minutes at room temperature. The supernatent solution was poured off into test tubes and frozen for storage.

B. Preparation of Indicator Spray

The substrate (15 mL indoxylacetate) was dissolved in 5 mL of absolute ethanol (solution 1). To make 13.0 mL of Solution 2, 4.0 mL of 0.05 M tris-(hydroxymethyl)aminomethane, 5.0 mL of 2M sodium chloride, 0.2 mL of 1M calcium chloride, and 3.8 mL of water were mixed. Solution 3 was water containing 0.05M potassium ferricyanide and potassium ferrocyanide. Just prior to spraying, the indicator spray solution was made up by mixing 13 mL of Solution 2 with 2 mL of Solution 3 and 5 mL of Solution 1, in that order.

C. Procedure

Standards (0.1, 0.01, 0.001 micrograms of agent per mL) and unknowns were applied to silica gel sheets. The spots were kept as small as possible and air dried.

The thin-layer sheets were then placed in a developer tank (ascending) containing a solution of n-hexane, acetone, and toluene (5:1:1). The mobile liquid phase front was allowed to ascend the chromatogram for 10 to 20 minutes to a height of 10 to 15 cm. The sheet was then removed and air dried.

The chromatograms were sprayed with a 1:5 mixture of the beef liver homogenate and 0.05 M tris-(hydroxymethyl) aminomethane buffer solution. The reaction between enzyme and inhibitor was allowed to proceed during an incubation period of 15 to 30 minutes in a constant temperature chamber (25 ^{o}C). EA 2192 must be incubated for at least three hours at this temperature; GB was incubated at low temperature (approximately 7 ^{o}C). The chromatograms are then air dried.

A freshly prepared indicator spray solution of indoxyl acetate was sprayed on the chromatograms. The sheets were observed closely for appearance of white spots against a blue background and the white spots were marked as they become visible. The standard spots were compared to the unknown in order to gain an estimate of the amount of inhibitors present and to check the sensitivity of the procedure.

D. Results

VX was separated from EA 2192 using this method. The RF for VX (relative to the solvent front) was 0.75 and zero for EA 2192. GB never appeared when the thin-layer sheet was placed in the developing solvent. It was only detected at the origin when the chromatogram was not developed in the solvent.

B.2.3.2 Detection of Agent Decomposition Products

The decomposition products KK, YN, and YL had a lower-detection limit of <1 μg. The following color-developing sprays used with these compounds were optimized for sensitivity.

A. Preparation of Indicator Sprays

An indicator spray of 0.5 percent bromocresol green in ethanol was used to detect YN and YL. This spray produced yellow spots on a green background if YN or YL was present in amounts greater than the lower detection limit.

The bromocresol green spray was also used to detect KK. However, the application of bromocresol green spray was followed by spraying with a 0.25 percent solution of rhodamine B in 2-propanol. These indicators produced a purple spot on a dark pink background. UV light also can be used to locate the spots if the concentration of KK in a sample was near the lower detection limit.

B. Procedure

Silica gel plates which have been activated at 100 degrees ^{o}C for 30 minutes were used for YN, YL, and KK.

The chromatogram used to detect KK was developed in a n-hexane, acetone, and toluene (5:1:1) mobile phase (this was also used for VX and EA 2192). The chromatogram was developed for approximately 20 minutes. The RF of KK was 0.40 relative to the solvent front. RF is the ratio of compound movement compared to the developing solvent.

The chromatogram used to detect YN and YL was developed in a 2.5 percent solution of acetic acid in acetone. The chromatogram was developed for approximately 20 minutes. The two acids moved only a short distance from the origin, but far enough to be separated from much of the extraneous materials present in environmental samples. The RF for YN was 0.12 and for YL it was 0.15.

B.2.4 LIQUID SCINTILLATION METHODS

The total radioactivity in plant extracts was measured using a Nuclear-Chicago Mark II liquid scintillation counter. The scintillation "cocktail" was prepared by dissolving 6 g of 2,5-diphenyloxazole (PPO) and 0.075 g of p-bis[2-(5-phenyloxazoly)]-benzene (POPOP) in scintillation grade toluene. The samples were prepared for counting by mixing 15 mL of the "cocktail" with two mL of the extract. If a cloudy solution resulted (due to the water extracted from the plants), Biosolve was added to clarify the sample.

A vial of 14 C 2-propanol (specific activity 250 μCi) was washed into scintillation grade toluene. Counting standards were prepared from this solution by diluting aliquots to the desired number of counts (activity).

Plant extracts, especially leaf extracts, are highly colored. Little quenching of the scintillation was observed in extracts from roots and stems. However, extreme quenching occurred with some of the leaf extracts. Attempts to remove the colored material from the samples (through the use of peroxide etc.) were unsuccessful. As a result, quench curves for leaf extracts were prepared by extracting different weights of leaf with 2-propanol and diluting two mL aliquots of these extracts in 15 mL of the "cocktail". This permitted correction of the observed scintillation results.

B.2.5 RADIOAUTOGRAPHY

Radioautographs of plants exposed to isotopically labeled agent were prepared to provide a visual means of determining the distribution of radioactivity within plants. At the end of the desired agent exposure period, the plant was removed from the contaminated nutrient, the roots were washed free of residual agent, blotted dry, and placed in contact with Kodak No-Screen medical x-ray film. The plant and film were placed in a

light-tight holder and stored for four to five days at -20 ^{o}C to minimize metabolic and hydrolytic processes (due to the low specific activity of the isotopically labeled GB used in these studies, a long exposure period was required).

APPENDIX C

2.3 GLOSSARY OF CHEMICAL CORPS SYMBOLS

GB	O-isopropyl methylphosphonofluoridate
IMP	O-isopropyl methylphosphonic acid
KK	bis(2-diisopropylaminoethyl) disulfide
KN	2-diisoproplyaminoethylthiol
QL	O-ethyl-O-(2-diisopropylaminoethyl) methylphosphonite
VX	O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate
WJ	O, O'-diethyl methylphosphonate
YL	O-ethyl methylphosphonic acid
YN	methylphosphonic acid
EA 2192	S-(2-diisopropylaminoethyl) methylphosphonic acid

APPENDIX D

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